

Neglected points of policy

The surreptitious way in which the British Government chose to reveal details of its science budget for 1984 suggests a desire to avoid awkward questions. But questions there must be.

ONE of the established conventions in British politics is that if a government is required to make public some decision which it would prefer not to see widely discussed, it does so by means of what is called a "written" answer to a parliamentary question at the very end of a parliamentary session. That way, Members of Parliament learn what has been said only if they diligently read the printed records of their proceedings sent to them by mail. This is how, last week, the Department of Education and Science dealt with the final allocation of the science budget for the coming financial year (beginning next April) to the handful of agencies that depend on it, principally the five research councils. So what, on this occasion, can the government have sought to hide? The figures (see page 723) are in themselves no great surprise; the only noticeable departure from the pattern of spending laid down last year by the Advisory Board for the Research Councils (ABRC) is that the Fellowship of Engineering (a would-be academy of engineers that, for the time being, functions as a pressure group for the doctrine that engineering has nothing to do with science) becomes a pensioner for the first time, with a grant of £150,000. It is unlikely that the House of Commons would have made trouble if this information had been made public in a more seemly way, from which it follows that the most probable motive for avoiding public discussion of the science budget must have been the wish to avoid giving reasons for what has been decided.

This is an unwelcome change from last year, when the British Government took its courage in its hands and actually let ABRC make public the reasons behind its recommendations on the same day that those were published. Then, it seemed, the objective was to demonstrate that decisions vitally affecting the pattern of publicly supported research had been rationally arrived at. While there is no reason to suppose that this year's decisions are irrational — and there is even talk that ABRC may make *something* public early in the new year — the awkward questions are qualitative different from what they were a year ago. There are five issues that the British Government should face.

Protection

First, the question necessarily arises of the sense in which the British Government has kept its promise that the science budget would be "protected". Since the Prime Minister, Mrs Margaret Thatcher, made the promise public four years ago, the funds made available for civil research have indeed remained numerically constant in spite of serious pressures on public spending. That is to the government's credit. But in the past few years, the resources the research councils in particular could spend on research have been declining for other than monetary reasons. The pace of inflation in research is not adequately measured by the general retail price index, the falling value of sterling has artificially increased the cost of overseas operations, commitments to particular lines of enquiry (such as information technology) have eaten into what is available for untied research while the recent belated discovery that too much of British public spending on civil research is tied up with internal laboratories means that the science budget will be robbed to pay pensions to people made to retire early ("restructuring" is the euphemism). With the decline in what universities spend on research support well under way, the promise of "level funding" is a myth.

Second, the British Government's conception of what its

advisory board is for is increasingly a mystery. For three of the past four years, committees set up to examine the way in which research policy is conducted in the United Kingdom have echoed one another in the plea that there should be some mechanism for overall consideration of the issues that affect the whole of the research enterprise. The government has stoutly resisted on the conflicting grounds that such a mechanism is not necessary and that, in any case, the Prime Minister is equipped to do the job. Attempts at formal planning of the research enterprise would indeed be disastrous: pluralism is best. But even the new ABRC, to which extra members from outside have recently been appointed and which has been asked to be more positive, appears not to be able to hammer out a policy on questions such as the relationship between public research institutes and universities without provoking complaints that the statutory autonomy of the research councils is being infringed. That cannot last.

Third, there are important questions to be asked about the proper balance between direct and indirect support for academic research. The science budget now carved up for next year is the direct component of support. The rest, once thought to be an almost equal amount (but which included an allowance for the salaries of academics in respect of the time spent on research) consists of what universities can spend out of their straitened budget or recruit from other sources, industry perhaps. In round numbers, the research councils between them spend £500 million a year, not a negligible amount. Over the years, they have been zealous in the pursuit of fairness in what they do. Applications for research funds are looked at with great care, at least if the time spent before decisions are reached is any guide. Peers review them, committees consider them and even if an application for funds is eventually turned down, the authors of frustrated projects may be invited to take comfort in the knowledge that in normal times their proposals would have succeeded. The whole procedure is probably as free from prejudice as it could be, but the price of fairness is rigidity, even dullness.

Fourth, these questions are far from being debating points to raise with a government so preoccupied with seemingly grander questions that it has only a little time to give to them. Sooner or later (on recent form, the second) decisions will have to be made about the future pattern of university support. The University Grants Committee has given itself until the early summer to digest the answers to the questionnaire sent out a few weeks ago. If the committee finds a way to encourage diversity among universities, it will also have a strong claim on part of the science budget. (If it fails, it will probably go out of business.)

Fifth, the issue of whether there should be more effective oversight of what the British Government spends on supporting research is emphatically neither a question of whether there should be central planning (which is impossible) nor of central direction (which would be intolerable), but rather whether there should be a mechanism for tackling large issues of general policy as they arise. As things are, the government's policy on research is not managed but instead allowed to stagger from one overdue upheaval to another. And all this is done in separate compartments, with different ministries following often contradictory policies and with defence research as stolidly isolated from the rest of what is done in Britain as it has ever been, the Prime Minister's promise last September of reform notwithstanding. □



Graduate balancing act

With industry poaching the best science graduates, US universities face a dilemma

FAMILIARITY more often breeds indifference than contempt. That is the chief lesson to be drawn from this week's report from Washington (see opposite) of the latest wave of alarm about the condition of graduate education in the United States. It is now well over three years since a joint commission of the National Science Foundation and the then Department of Education spelled out the problems that have since become still more apparent: shortages of able teachers in fields where universities cannot compete as employers with industrial corporations, surpluses of teachers in fields (mostly in the arts and humanities) where students are now in short supply and a general lack of resources with which to ensure that even where teachers and students are appropriately matched, graduate education and research can be prosecuted as effectively as they might be. Such palliatives as there have been, the decisions separately by the National Science Foundation and the Pentagon to spend money on university equipment, and the tax relief offered to corporations supporting academic research, cannot by themselves be a remedy.

Electronics, computer science and related fields of study should provide the exemplar of a solution. The rapidly growing electronics industry relies on academic graduate schools for recruits to its growing labour force but is also predatory on university faculties whose members are potential employees of a particularly valuable kind. While even the richest universities cannot compete with the corporations in the direct monetary rewards they are able to offer, well-established academics are kept at their posts by the inherent interest of the jobs they do and by the opportunities that arise to supplement their academic salaries by consultancy. The rub comes with less well-established people, or with those who happen to work at universities whose reputations are not among the highest.

For what it is worth, the squeeze on the training of skilled people does not seem so far to have created insuperable problems. But there has not yet been time for the more subtle but perhaps more important difficulties to become apparent. How many of those now being hired will be able to contribute to their employers as fully as they might (and as their salaries suggest they should)? And how many of them have been sufficiently well-taught at graduate school to be able to hold their own in an industry most of all distinguished by its competitiveness? Prudent people thus affected will no doubt recognize the risks and may even reconcile themselves to periodic returns to graduate school during their careers. There is a case for asking that employers should more often than at present recognize the importance of what the graduate schools have to offer people in mid-career.

But who will teach? If too many academics have fled to industry, the graduate schools may even be too ill-equipped to undertake such important tasks. The simplest solution is that corporations in the shortage fields should follow the pattern common in Japan, making arrangements with university departments that will enable the departments to contribute directly to industrial development by means of formal development contracts. The result, in the short run at least, could be beneficial for both parties to these contracts and for graduate education as well. The snags, also evident in Japan, are that if such arrangements become permanent, or even too common, academic life must in the long run be damaged. University presidents looking to such bilateral arrangements to keep hard-pressed departments alive should be conscious of the dangers.

More serious difficulties arise in more traditional fields of study, where the pace of technological change is not nearly as rapid but where the need for trained people is still as great as ever. Many university departments of chemistry in the United States now find themselves less able than in the past to attract able graduate students, perhaps because the most able to have rushed off in other directions, perhaps because the traditional assump-

tion that a graduate course would carry with it an opportunity to join a university faculty is now recognized to be an illusion and perhaps because of the much-publicized consequences of the recession for the industry. The chances are that potential graduate students have over-reacted to the changing pattern of the real world in which they will eventually have to work. The irony is that the future well-being of these industries may be jeopardized both by the fall in the numbers of graduate students being trained and also by the danger that the pool of academic research itself will shrink. These are the fields most in need of direct help from outside — from university administrations and from grant-making bodies such as the National Science Foundation. The modest sums so far spent on equipment can only scratch the surface.

The future for other fields of study is even more bleak. In the arts and the humanities, universities in the United States have been under pressure for the whole of the past decade. Now, for some of them, the pressure has become intolerable. Except in vocationally oriented fields of study such as law, there has been a decline in number of undergraduates as well as graduate demand. The result is that outside the major universities, faculty strength is being run down as quickly as can be managed. In the short run, universities have no alternative; in the long run, the result may be that universities are unable to provide the range of undergraduate teaching that society expects of them. Their capacity to provide a general education for undergraduates will be impaired, as will be their contribution to scholarship at large. The only effective remedy lies with the federal government, which should quickly increase the small pot of money from which research in the humanities is at present supported. The march by students towards fields of study thought to be useful in the job market, which will be welcomed in Washington, requires that the federal government should take steps to support the fields from which the stampede is taking place. The hope that Washington will see the problem in this way is, unfortunately, only small. □

Southern comfort

If Argentina becomes nuclear-free, will Brazil follow suit?

NOBODY will be surprised that the new government in Buenos Aires is less keen than its military predecessors on making nuclear weapons, but the report of what has been said in Argentina (see p. 724) since last month's elections is encouraging. Since the late 1950s, Argentine governments have been blowing hot and cold about their plans for nuclear energy, apparently unabashed by the discovery that the first plan to harness thermonuclear fusion for purposes of all kinds collapsed in confusion and allegations of fraud. Since then, there has been a loose but far from perfect correlation between the military stripe of a government and its nuclear ambitions. (The first Peron government, which might have been expected to renounce military plans for nuclear energy, was instead studiously ambiguous.) More recently, the Argentine Government position has been an amalgam of the arguments that nuclear energy is economically essential and that signature of the Treaty of Tlatloco, which would make South America a nuclear-free zone if only Argentina and Brazil would join, would be politically compromising. So it will be interesting and important to see whether the time is now ripe for Argentina to take the plunge and for Brazil, contrary to rumours, to follow.

No country in South America can possibly have a serious interest in nuclear weapons. The political cost to a country which acquires them would consist not merely of deteriorating relations with near neighbours but the risk of intervention from outside. In a region where political boundaries have been uncannily stable while governments have come and gone, everybody would be the loser. The snag, hitherto, is that Argentina appears to have been unable to join the treaty because Brazil has stood out against it — and that Brazil has followed exactly the same line. Is it now time for other South American governments (nobody from outside could help) to put pressure on the two laggards? □

US universities

New crisis looms for graduate education

Washington

GRADUATE departments in US universities face severe shortages of talented doctoral candidates in areas such as engineering and computer science, while good staff are frequently lured away by the better pay and newer laboratories available in industry. These are the principal findings of the report last week of a commission appointed jointly by Congress and President Reagan.

The commission found what it describes as "startling inadequacies" in the number of doctoral students entering important fields. In 1979, for example, only 309 doctorates in chemical engineering were awarded in the United States, and nearly half of those went to foreign students. The dwindling number of new doctorates is making it impossible for some universities to replenish depleted faculties in fashionable fields such as computer engineering, solid-state electronics and digital systems.

In other fields, however, the problem is reversed. Shrinking employment opportunities in the humanities and social sciences have reduced staff turnover and blocked the careers of younger academics. Many young humanists and social scientists, the commission says, now lead lives of "quiet desperation", making up a class of itinerant scholars wandering from campus to campus in the often vain hope of securing a tenured post.

The collapse of the academic job market has discouraged many graduates from pursuing academic studies. A telling statistic reported by the commission is that only a third of those who graduated with a good degree in 1980 planned traditional graduate studies compared with more than three-quarters in the 1960s. Many of the best graduates were more inclined to switch to professional studies in law or medicine.

Science graduates who do choose to pursue a career in research do not always find that a university offers the best research environment. Surveys have shown that university equipment is usually twice as old as that in leading commercial laboratories, prompting a university respondent to a survey by the Association of American Universities to complain that the ivory towers are now in industry.

Thomas Kailaph, associate chairman of electrical engineering at Stanford University, told the commission that a \$13 million engineering equipment fund set up by the Department of Defense had been swamped by requests totalling more than \$1,000 million. The University of California estimates that since 1975, when 25 per cent of its research equipment was

obsolete, obsolescence has claimed an additional 5 per cent each year, while state appropriations for equipment replacement have averaged less than 2 per cent of replacement costs.

Something is being done, however. The commission applauds the National Science Foundation's plan to increase its support for equipment and instrumentation from an estimated \$90 million in 1982 to \$180 million by 1984. The Pentagon is also increasing its spending on university research facilities. But the commission calls for an even greater effort and wants

Congress to approve new methods, such as larger tax incentives and federal matching grants, to persuade industry to donate equipment to universities.

The commission also recommends a substantial increase in federal support for research fellowships and assistantships. Graduate students, it says, should remain eligible for the government's Guaranteed Student Loan scheme, which enables students to borrow up to \$5,000 a year on a fixed interest rate of 8 per cent, with repayments postponed until graduation. Nearly half a million graduate and professional students used the loan scheme in 1982, and although the Reagan Administration, concerned about the mounting cost, proposed restricting the scheme to undergraduates, it later dropped the proposal when faced with strong congressional opposition.

Peter David

UK nuclear power

Possible prosecution for Sellafield

THE Director of Public Prosecutions is this week considering whether there is a case for criminal proceedings against British Nuclear Fuels Limited (BNFL) over last month's accidental radioactive discharge into the sea from its fuel reprocessing plant at Sellafield (formerly Windscale) in Cumbria. A preliminary report into the incident by the Nuclear Installations Inspectorate is being studied, and could lead to prosecution under the Health and Safety at Work Act, while a separate report by the Department of the Environment, now being considered by ministers, could form the basis of a prosecution under the Radioactive Substances Act.

It is clear that the discharge was larger than BNFL at first admitted. In addition to some 600 curies of beta radioactivity in aqueous solution, an unknown quantity of particulate matter and chemical solvent was released. BNFL says the total amount of radioactivity released was substantially less than 4,500 curies and so within authorized limits.

The accident occurred because of a "misunderstanding" between plant managers. On 11 November automatic alarms were triggered when water, solvent and "crud" were drained into a sea discharge tank before the highly radioactive solvent and "crud" had first been separated off. The flow was stopped, but not before 4,500 curies had found its way into the sea discharge tank, intended only for dilute aqueous waste. The contents of the tank were eventually discharged into the sea through a single 2-inch diameter pipe. Mr Con Allday, BNFL's chairman, said last week that the sea tank was not meant to be a last barrier before the sea and that management decisions after the original error had been correct. Staff had acted at all times in good faith; nobody had been sacked or suspended from duty.

The discharge was first brought to public attention by Greenpeace, the "direct action" conservation group, after radioactive contamination of their divers on 15 and 16 November and BNFL's first public statement on the matter was made on 19 November. The statement referred only to 500 curies of beta radioactivity in aqueous solution, and made no mention of the particulate matter, which, sticking to seaweed and marine debris, led to a public warning by the Department of the Environment against use of beaches near Sellafield.

Last week Mr Allday accepted that the nuclear industry had to be, like Caesar's wife, above suspicion, and that BNFL had fallen from the high standards rightly expected of it. But he stressed that there was no evidence that the public had been put at hazard as a result of the accident. He also complained that BNFL had not received due credit for the action it had taken to reduce sea discharges from the high levels of the early 1970s. Needless to say, operating procedures have been revised to prevent a repetition of last month's accident, even though it was probably not an important health hazard.

Perhaps of more concern are the alpha-emitting actinides (especially plutonium), which are discharged routinely and bind to sediments on the sea bed. Safety limits for plutonium were effectively lowered by a factor of fifteen recently (see *Nature* 15 December, p.634). The National Radiological Protection Board announced this week that it is to set up a controlled investigation into household dust as a possible exposure route for actinides, which are resuspended and concentrated from sediments and then blown ashore in sea spray. But, says the board, exposure through this route does not at present appear to be a significant hazard.

Tim Beardsley

Weapons research

Sandia's nuclear simulation

Albuquerque, New Mexico

A NEW \$39 million laboratory that will significantly increase the ability of nuclear weapons designers to simulate the effect of radiation on critical nuclear components is under construction at Sandia National Laboratories. The centrepiece of the new laboratory is a 20 trillion (20×10^{12}) watt gamma-ray source to simulate the intense short pulse of radiation from a nuclear explosion that can disable the electronics necessary for arming and fusing nuclear weapons as they approach their targets. A 16.5 trillion watt X-ray source will also be available at the new Simulation Testing Laboratory through the recycling of Sandia's Pulsed Beam Fusion Accelerator (PBFA-I), at present used for inertial confinement fusion research. The laboratory will be completed in 1987.

Sandia, which has responsibility for the engineering of nuclear weapons, has been heavily involved in the development of "hardened" electronic components. This work has been prompted by concern that the credibility of the United States deterrent would be lost if its nuclear weapons were vulnerable to counter-measures such as the preemptive detonation of a nuclear bomb. The technical challenge in simulation testing has been to create in the laboratory a pulse of gamma and X radiation that is both intense and short. Conventional pulsed power technology faces an intrinsic obstacle: methods used to increase current also tend to increase inductance and pulse length.

Sandia's solution, which will permit its new gamma-ray machine, Hermes-III, to produce a pulse of 1 million amperes at 15 million volts in a mere 20 nanoseconds, involves a technology that has implications for controlled fusion, nuclear weapons design and also particle-beam weapons — all of which are intimately wrapped together in the laboratory's pulsed power research (see below). The technique is to split the pulse generator into small nodules, which are combined in series or parallel to yield either high voltages or high currents while keeping inductance small.

The result is that Hermes-III will produce 10 times the power of the best single-module machine (Hermes-II, built in 1967, and still Sandia's work-horse for gamma-ray simulation) in a pulse less than one-tenth as long. PBFA-I, when adapted for X-ray simulation, will offer a similar improvement over the laboratory's present X-ray simulator.

The multiple-module concept was first applied in the Department of Defense's "Aurora" machine, which runs four pulse generators in parallel to produce a high current. Sandia then built a series of machines improving on this multiple-module principle. As well as their use in X-ray simulation, they also paved the way

for particle-beam fusion research, which PBFA-I was designed to explore. PBFA-I uses 36 parallel generators arranged like spokes of a wheel, converging on a target at the hub. It generates 15 million amps at 2 million volts.

Although the combination of high current and short pulse offered by the parallel module arrangement is equally applicable to gamma-ray production, high voltage is needed also to generate the more energetic gamma rays. Gerold Yonas, director of Sandia's pulsed power research, says that in 1975 a Soviet publication suggested the possibility of tying modules together in series to produce such high voltages (of the order of 10 MV are needed). Sandia conducted a joint project with the Air Force Weapons Laboratory in 1978 to prove the feasibility of the concept, which in effect involves creating a linear accelerator out of the modules. That is the technology that will be utilized in Hermes-III and which is now being further developed in a test-bed called MABE.

Sandia scientists are quick to insist that

in spite of these improvements, laboratory simulation will never be a complete substitute for testing with actual nuclear explosions, as now carried out at the Nevada test site. Yonas says that the extrapolations of the laboratory tests to the actual spectral distribution of radiation produced in a nuclear blast and to the actual pulse and decay rates are necessarily problematical. But the actual insistence on "proof-testing" under the more realistic conditions at Nevada may have more to do with nuclear strategy than scientific certainty. "Deterrence is nothing but peception", Yonas says. "Ultimately, the credibility of our deterrence lies in a proof test."

Laboratory simulation does permit a more controlled approach to understanding radiation effects on electronic components; it is also cheaper than setting off nuclear explosions and can be done much more often. Sandia's new facilities will be used chiefly to test the radiation hardness of nuclear weapon electronics; the laboratory also expects to continue a modest amount of contract work for the Department of Defense, testing electronic systems in battlefield equipment.

Stephen Budiansky

Mixed mission brings stability

SANDIA National Laboratories, founded in 1945 to provide engineering support for the Manhattan Project at Los Alamos, continues to be chiefly concerned with weapons research, development and testing. Its increasing strength in basic science, however, particularly in the pulsed power research programme, has given the laboratory something of a split personality.

The entanglement of interests is perhaps most apparent in the inertial confinement fusion (ICF) programme at Sandia. Begun in the 1970s, the ICF programme was at first justified chiefly by the promise of commercial fusion energy. The programme was, however, always supported by the office of military applications at the Department of Energy and its predecessor agencies. And now that ICF has lost its bloom, with magnetic confinement fusion emerging as the more practical route to commercial energy production, its status is even more confused.

A new ICF machine, PBPA-II, which will combine the parallel and series arrangement of modules to produce 100 trillion watts at as much as 24 MV in a pulse 13 nanoseconds long, is in many ways designed with controlled fusion research in mind. The decision to go to higher voltage was prompted by the choice of lithium ions rather than protons for the particle beam, which is focused on a deuterium-tritium pellet, lithium ions can be more easily focused, giving greater power density.

On the other hand, programme-director Yonas concedes that "our choices have been influenced primarily by our short-

term goals," which include studies of pellet shapes with significance for nuclear weapons design. PBFA-II will, however, be unclassified, except when a "classified pellet" is being tested.

The simulation programme has similarly been intimately wrapped up with the ICF research and the same may happen to so-called "star wars", or particle-beam weapons. Although Yonas says that PBFA-II "would be a loser" for particle beam weapons research, the basic principles of pulsed power are clearly applicable.

Sandia's combination of a primary weapons mission with a broad expertise in energy sciences and microelectronics has been a formula for financial stability. When the Reagan Administration cut energy research by 40 per cent, the laboratory's executive vice-president Albert Narath says, Sandia had little trouble shifting affected staff members to defence research.

The laboratory's current budget of \$874 million (with 8,087 full-time employees) has remained fairly level in recent years, with 60 per cent devoted to nuclear weapons activities. Narath thinks there may be a major shift if the "star wars" initiative materializes: "We are thinking very hard about the possibility of transitioning to a defence-dominated deterrence." Sandia's expertise in pulsed power and in hardened microelectronics would place the laboratory in a "very good" position for contributing to a star wars initiative, says Narath.

Stephen Budiansky

High-energy physics

New collider gets the nod

BRITISH high-energy physicists were hardly able to believe their luck last week. The ruling council of the European Organization for Nuclear Research (CERN) at Geneva approved an upgrading of its proton-antiproton collider that will cost SF55 million (£18 million) despite the insistence of Britain that it could not provide its share of what amounts to a loan to CERN to cover the cost of the project. British scientists are therefore looking forward to using significantly improved facilities at no extra expense.

The decision taken last week was to upgrade the antiproton beam by building an antiproton collector, ACOL, to supplement the existing antiproton accumulator. The collector, which fits in an existing building, will accept and stochastically cool antiprotons with a wider angle and momentum range than the existing accumulator, thus increasing intensity. A new lithium lens downstream of the metallic target that produces the antiprotons is also planned. The upgrading will lead to at least a 10-fold increase in luminosity (events per second) and a modest increase in energy, from 270/270 GeV to 310/310 GeV. The improvements will ensure that the collider, which generated much excitement earlier this year for producing the first evidence of Z^0 and W bosons, stays competitive for an extra 3 or 4 years, well into the 1990s.

The problem facing last week's council meeting was that CERN's budget is already fully committed. The suggestion made was that member states might temporarily forgo about one half of a special rebate that has become payable this year with the accession to the organization of Spain. The rebate would instead be made upon the completion of LEP, CERN's electron-positron collider now under construction. According to CERN's rules, late joiners have to make "special contributions" in addition to their normal subscriptions, in order to compensate existing members for capital they have already invested. ACOL, it was argued, could be built with part of Spain's "special contribution".

Britain, however, was adamant that, because of domestic financial pressure on the Science and Engineering Research Council, it would not be able to accept any postponement of the rebate. Other major contributors (France, West Germany, Italy) were able to be more flexible, perhaps because, by CERN's budget rules, their contributions (unlike Britain's) are set to decrease in future years owing to their relatively poor economic performance against the Swiss franc.

According to Dr John Walsh of the British Science and Engineering Research Council, who was a member of the UK delegation, other countries are unlikely to want to enforce restrictions on British

scientists using the upgraded collider, which is the only one of its type in the world and will remain so until the Fermilab Tevatron comes on stream later this decade. Financial details of the ACOL package will be settled at a meeting of CERN's financial committee in February.

Tim Beardsley

UK science budget

The pie is sliced

SIR Keith Joseph, Secretary of State for Education and Science in the British Government, last week gave notice of how the £549 million his department intends to spend directly on scientific research next year will be allocated between the five research councils.

The total available for the science budget has been known since last month (see *Nature* 24 November, p.304). Within this total, Sir Keith had very little room to manoeuvre without bringing down on himself the wrath of one or other of the councils, and the results of the exercises are broadly as foreseen a year ago in a forward plan published by the Advisory Board for the Research Councils.

The lion's share of the science budget goes to the Science and Engineering Research Council (SERC), which is scheduled to receive £278 million next year. This total includes £6 million intended to help SERC meet the cost of its obligations in subscriptions to international organizations (mainly the European Organization for Nuclear Research), rather less than the £10 million or so that SERC reckons it is lacking on this score. But negotiations on future allowances for subscription costs are continuing and SERC hopes that an annual sum will in future be reserved for the purpose. The Medical Research Council will receive £119 million, a slight reduction on what had been expected, and the Social Science Research Council is to be drip-fed on £22 million. In the middle league are the Agricultural and Food Research Council (AFRC) and the Natural Environment Research Council (NERC), which will receive £46.5 million and £65.0 million respectively. NERC last year received an unexpected boost in the form of special support for research in the Antarctic, and this is to continue next year. But neither NERC nor AFRC will receive any significant special allowances for the costs of their enforced restructuring. Like AFRC (see p.725), NERC will now have to face up to a drastic realignment of its research priorities. Dr K. Aldred, the council's finance officer, says that it is impossible to put a figure on the cost of restructuring that the council faces, because many proposals are dependent on the level of support allowed.

Tim Beardsley

Biotechnology

Celltech loses its MRC deal

BRITAIN'S government-backed biotechnology company, Celltech, is to lose its broad right of first refusal on the development of Medical Research Council (MRC) discoveries in recombinant DNA and cell hybridization — a right it has enjoyed for three years under what was originally a five-year agreement. The only rights it will retain will be in areas where collaborative research is already under way, or in a slightly broader area where Celltech already has plans to commercialize an MRC result.

In effect, this may restrict Celltech's rights almost entirely to hybridomas, which certainly puts the young company in a more bracing commercial environment. But, said chief executive Gerald Fairtlough on Monday, Celltech "has never taken anything for granted". Although "it wasn't planned" when Celltech was established that it might lose its exclusive rights, it was "a perfectly natural evolution". Moreover Celltech was gaining another five-year agreement with the research council, albeit on more restrictive terms. The new agreement, says Fairtlough, also specifies that while Celltech will have no general rights in recombinant DNA and cell hybridization, there will be "a general intent to work together". The existence of a framework of agreement over patent rights will also aid collaboration, Fairtlough believes.

Does the MRC now intend to hawk some of its research around competing companies? Fairtlough does not know or will not say. Others may have been inhibited in approaching MRC by the Celltech link, he admits. "But I hope we have done and will continue to do a good job for them."

Robert Walgate

● Meanwhile in France the Marseilles-based Immunotech announced last week the opening of new laboratories to house its 16 postdoctoral scientists and 12 technicians. Rather like Celltech, Immunotech has first refusal rights on some areas of research of the national medical research council. Immunotech's agreement with INSERM covers immunology, including vaccines, until 1990. The company's laboratories are built right on the doorstep of the largest immunology laboratory in France, jointly established in 1976 by INSERM and the Centre National de la Recherche Scientifique and directed by Professor François Kourilsky. Celltech, although doing business in France, has not yet felt the winds of competition from Immunotech, which like the British company deals with both contract research and product development (it has 30 monoclonal antibodies on the market). Immunotech is not profitable yet but expects to go into the black in a year or two, says scientific director Michel Delaage. □

Nuclear weapons

Argentina backs away

PRESIDENT-elect Raul Alfonsín of Argentina, as one of his first acts, has ordered the drafting of a new law to control the country's nuclear energy programme. Foreign minister-designate Dante Caputo, speaking on Buenos Aires radio, said that the purpose of the law will be to study the organization of the National Commission for Atomic Energy (CNEA) "so that its activities may be in keeping with the policy of using nuclear energy for peaceful purposes". Mr Alfonsín has decided that CNEA should be "directly subordinated to the executive branch". The foreign minister, the presidential secretary-general and a presidential adviser will reorganize the structure of CNEA and work out a mechanism for the legislative control of the implementation of the government's peaceful nuclear policy.

The stress on Argentina's exclusively peaceful nuclear aims seems intended to allay international fears that the development of the gas-diffusion uranium enrichment plant at Pilcaniyeu might have sinister implications — particularly in view of the strict secrecy that has surrounded it.

According to the outgoing CNEA chairman, Vice-Admiral Carlos Castro Madero, this secrecy was necessary because of the "very tough policy against nuclear proliferation" inaugurated by the Carter Administration in the United States. Had the Argentines revealed that they were planning an enrichment facility of their own, they could have been blocked by an embargo on the supply of essential equipment. Castro Madero further hinted that secrecy had also been necessary because there would have been considerable scepticism that Argentina could succeed in such a project — and any delays and setbacks could have meant loss of face for CNEA. As for the idea that Argentina might make an atomic bomb, he told a television interviewer, that would be "the most absurd thing", wasting human and financial resources and "provoking other countries" to do likewise.

But Vice-Admiral Castro Madero has not always held that view. In 1980, he claimed that "with a little help", Argentina could have its own nuclear bomb. In April 1983, he commissioned a feasibility study on the construction of a nuclear powered submarine for the Argentine Navy (while admitting that the ultimate decision must be taken by the government and people of Argentina).

His resignation, shortly after the election of Mr Alfonsín and the announcement of the completion of the Pilcaniyeu project, raised international hopes that Argentina might soon decide to accede to the Non-Proliferation Treaty.

Ironically, as fears of an Argentina nuclear bomb fade, rumours have begun to circulate that Brazil is working on such a

bomb. According to the newspaper *O Estado de São Paulo* of 9 December, Brazil will have "total technological independence" in seven years, and will concentrate on a "strategic" 20 to 30 kilotonne plutonium bomb, to be carried by a missile with a 3,000-km range. Commenting on the rumours, Waldir de Vasconcelos, chief of staff of the Brazilian armed forces, said that Brazil would research the "entire potential" of nuclear technology.

"Any country that wants to progress must carry out that research", he said, "and whoever manages to master nuclear technology can even manufacture a bomb." The production of a nuclear bomb would, he stressed, be a political decision, but he did not say whether such a decision had been taken.

Vera Rich

Minister recants

A PUBLIC apology was made last week by Jerzy Urban, the Polish Government's chief press spokesman, to the "BBC correspondent" in Warsaw for an "unjust attack" made a few weeks earlier, in connection with the documents which Polish scholars must sign before they can travel abroad.

Mr Urban's "unjust attack" was quoted on 5 December by the government daily *Rzeczpospolita*. Urban went on to say "Furthermore, the British press carried information, broadcast also by the BBC, that persons travelling abroad in fact undertake to present their attitudes in accordance with the *raison d'état* of the Polish People's Republic. And that such obligations have to be signed. This information is manufactured out of thin air."

On 13 December, however, Mr Urban said that "after further investigation of the issue it turns out that the research workers of the Polish Academy of Science who go on official visits abroad do, in fact, sign instructions which include such a sentence. My previous statement of last November was based on information from the Ministry of Science, Higher Education and Technology . . . I failed to notice that this ministry is not the only institution which sends research workers abroad, and thus made an unjust claim against the BBC."

Mr Urban (or his research team) has still, in fact, failed to get his facts right. Kevin Ruane, the BBC correspondent in Warsaw, had not, apparently filed the story at all. Mr Krzysztof Pszenicki, head of the Polish section of the BBC commented that he had seen no despatch on the subject from Kevin Ruane or from any other source, except for the report in *Nature* on 3 November, which was reviewed in the BBC Polish section's regular "Review of the Weekly Press".

Vera Rich

Information technology

Esprit foiled in budget impasse

BRITAIN and West Germany last week dealt a blow to Esprit, Europe's fledgling and belated effort to coordinate research and development in information technology. Esprit has been heralded by European industry as the best research plan ever to emerge from the Brussels-based European Commission, and it was due to receive first full funding next year; but after last week's meeting of European research ministers, it will get none, at least for the time being.

The problem stems from the earlier debacle of the European summit in Athens, where heads of state failed to agree on a permanent mechanism for reducing Britain's and West Germany's contributions to the Brussels budget. Net earners — notably France — from the Common Agricultural Policy (CAP) were notably reluctant to make concessions on CAP, and the net contributors refused to budge without such concessions. So Britain and West Germany refused to support Esprit until the "overall shape" of next year's budget is agreed.

"It was just too soon after Athens", said a British government spokesman. "No-one had had a chance to draw breath." However, the research council did resolve the last remaining problems on the management and structure of Esprit, so there is now a neat "Esprit package" just waiting for funds. These could be released before the end of January, Britain feels, provided Esprit can be politically disentangled from other European issues.

Brussels observers, however, describe the British and German attitude as illogical. They appear to be playing the "Esprit card" to help win concessions on other issues, it is felt, but the play might rebound, as both countries stood to gain more from Esprit than other partners because their relatively strong information industries were likely to take the leading role in many of the Esprit projects.

Moreover, Brussels research commissioner Etienne Davignon offered a way out. He argued that as all members were agreed that Esprit had the highest priority, the Commission could find ways to support it by cutting or delaying other work, but British and West German representatives would not agree to this proposal.

According to the Commission, Esprit will have to be abandoned if there is no agreement before June, when the pilot phase ends. Industry cannot wait longer, Brussels says. In Britain this view is said to be somewhat exaggerated, but industry is certainly going to find it difficult to understand that while the 10 nations are fully agreed on the programme, the money ("a flea-bite on a gnat's elbow", according to a Commission man) is not going to be released.

Robert Walgate

Agriculture research

Retrenchment plan launched

PUTTING a brave face on its sorry state of affairs, the Agricultural and Food Research Council (AFRC) last week launched its Corporate Plan, an outline of the financial expectations and research priorities for the Agricultural and Food Research Service (AFRS) for 1984 to 1988. The most drastic and immediate part of the plan involves, as expected (see *Nature* 8 December, p.523), the closure of AFRC's Letcombe Laboratory and the Weed Research Organization, and an overall loss of 250 jobs by next spring. Another 250 posts will have disappeared by 1986-87 even on the somewhat optimistic financial basis on which the plan has been drawn up.

Of the impending redundancies, about a hundred will have to be compulsory and about half will be scientific staff. Posts will be lost at most of AFRC's institutes. Some of the redundancies at Rothamsted Experimental Station are designed to make space for incoming members of the Letcombe Laboratory, whose research is to survive their institute's closure. Similarly, some of the staff of the Long Ashton Research Station are to make way for retained members of the doomed Weed Research Organization. These changes will save AFRC £2.4 million a year and represent a major cut-back in research on the growth and protection of arable crops.

Other areas of research that have been deemed to be over-supported include nutritional imbalance and trace elements in animals, infectious diseases of animals and the nutrition and production of cattle,

sheep and pigs. Each of these areas of research is to be cut back by £1 million a year. Lamenting the cuts last week, AFRC's secretary, Dr Ralph Riley, said it was distasteful that some excellent work of great relevance to food and agricultural production would have to be stopped, but said it was inevitable that some areas of research had to suffer if those identified as under-supported were to grow under current and projected financial constraints.

In its look ahead, AFRC projects an AFRS expenditure of £131 million by 1987-88, an apparent increase of 7 per cent over 1983-84 but a loss of 7 per cent in real terms, even if inflation is held to a modest 3-4 per cent per annum. The restructuring and redundancies have been worked out on that projection but, warned Dr Riley, more problems will emerge if, for example, pay increases to staff next year exceed the 3 per cent allowed for by the Department of Education and Science. Staff costs currently account for at least 60 per cent of expenditure but that should fall by a few percentage points by 1987-88.

In its corporate plan, AFRC confirms its intention to carry through two shifts in policy that have been strongly recommended to it. The first is a shift towards the support of food research, recommended by the Advisory Council for Applied Research and Development last year. In response, "food" has been incorporated in the title of the erstwhile Agricultural Research Council and expenditure on food science is

New company's plans

THE Agricultural Genetics Company (AGC), founded in July to exploit biotechnological discoveries in five institutes and one unit of AFRC, hopes to raise about £10 million from new and old investors and to build a laboratory in Cambridge during 1984. Acknowledging that UK government cutbacks in support of AFRC had helped create the climate for AGC's formation, Dr Roger Gilmour, its chief executive, implied last week that changes in attitude within AFRC towards the commercialization of its research must continue.

Speaking at the end of a two-day meeting convened by AFRC to review the first five years of its programme of genetic manipulation of crop plants, Dr Gilmour explained that a confidentiality committee had been set up to make rapid decisions on the commercial potential of discoveries reported in papers that AFRC scientists hoped to publish. Any papers that had to be suppressed to protect commercial interests would still be accredited to the authors' files. The first product of AGC, he said, would be improved inoculations of

Rhizobium bacteria, responsible for the nitrogen-fixing nodules formed on the roots of legumes.

Earlier in the meeting, Professor Joe Key of the University of Georgia, Atlanta, and a consultant to the US company Agrigenetics, had paid what he described as a back-handed compliment to the AFRC programme of genetic manipulation of crop plants. It was, he said, far better value than anything produced by the United States Department of Agriculture which was inflexible, unwilling to submit its programme to peer review and too concerned with the criterion of relevance. His plea that relevance should not dominate the choice of priorities in agricultural research reflected a similar plea from Professor Harold Woolhouse, director of the John Innes Institute who also urged that the problems of agriculture in developing countries should not be forgotten. On the same subject, Gilmour later said that fears that his company would ignore the needs of developing countries were overdone. In the first place AFRC had done nothing for them and in the second he was insulted by suggestions that businessmen did not have social consciences.

Peter Newmark

IRAS discovery

THE Infra-Red Astronomical Satellite (IRAS) has found another star that is surrounded by a dust cloud. The first, Vega, was discovered earlier this year and caused excitement because it was the first known example of a star (other than the Sun) orbited by solid particles, of size 1mm and greater. Such a cloud is thought to have surrounded the Sun early in the evolution of the Solar System. Vega, however, is expected to reach the end of its "main-sequence" lifetime before a planetary system could evolve — assuming the conditions were right for such an evolution.

The second such star, Fomalhaut, is similar to Vega in that it is a well studied main-sequence object that has turned out to be surprisingly bright at infra-red wavelengths. Fomalhaut is twelve times more luminous than the Sun and one fifth as bright as Vega. With a lower surface temperature than Vega (8,800 K compared with 9,600 K), its lifetime should be longer, although IRAS astronomers are as yet unable to provide numbers. Fomalhaut is 22 light years from Earth, and the dust shell is about 100 astronomical units (Earth-Sun distances) in diameter. Although IRAS itself has now ceased operation, the search for similar objects through its data banks continues.

Philip Campbell

projected to grow by 12 per cent (allowing for inflation) over the next four years, by then accounting for about 13 per cent of the total budget. The other shift in policy is towards more university research as urged upon AFRC by the Advisory Board for the Research Councils. Grants to universities should amount to £7.7 million by 1987-88 compared with £4.6 million this year. Much of the extra money will be spent on research on food and human nutrition and, where possible, the university research will be linked to and complement research within an AFRC institute. For example, Professor A. Keller in the department of physics at the University of Bristol is to receive an AFRC grant to carry out biophysical studies on collagen and other meat proteins to complement the biochemical research of the Meat Research Institute.

Dr Riley expressed hopes that the new emphasis on food research would prove to be an enticement to the private sector which would convert the research into benefits for the consumer while providing an additional source of income for AFRC, perhaps in a way parallel to the formation of the Agricultural Genetics Company (see alongside), although no substantial revenue is anticipated from that source by 1987-88. Asked whether the promise of revenue from the private sector would not undermine the council's commitment to basic research, Dr Riley said he was unable to deny the possibility but felt the effect was never likely to be a major one.

Peter Newmark

Franklin Valley sites

SIR — Dr S.J. Paterson accuses me and others of lacking “scientific objectivity” (*Nature* 29 September, p.354, but see also *Nature* 15 December, p.636) in our involvement in the recent Australian High Court case over construction of the Franklin dam in the south-west Tasmanian World Heritage area. He quotes me as saying that “it is most unlikely that sites providing material of comparable archaeological value” would be found in south-west Tasmania and goes on to claim that such sites have been found.

Part of the Tasmanian legal strategy was to contest any statement that ascribed special cultural or natural value to the area, which is why the Geological Section of the Hydro-Electric Commission (HEC) conducted its own search for archaeological cave sites in May 1983. This two-week operation cost \$93,600 in a state which had not spent a third of that amount on prehistoric field research in the previous ten years. Moreover, the responsible Tasmanian government department, the National Parks and Wildlife Service, which has five qualified archaeologists on its staff, was ignored, as were the procedures for obtaining permission for archaeological research specified by the Aboriginal Relics Act (1975).

No archaeologists were involved. Dr Paterson says that the Association of Consulting Archaeologists had warned its members not to take part but the plan was to dig out the entire deposits of the caves, a procedure that many archaeologists believed was professionally unethical and indeed contrary to the principles of the ICOMOS charter. In any case, very few academic archaeologists in Australia are members of the consulting group, and no archaeologist that I know of was approached to assess the new claims.

Dr Paterson's claim that his team found in different valleys five caves “with content similar to the Franklin Valley caves” is interesting but cannot be assessed while none of the data are published. I note, however, that of his sites, Site 6 on the Andrew River would have been drowned by Stage 2 of the proposed integrated power scheme and that Site 9, Nelson River cave, had already been published six months before the HEC search began and that the only claim for a possible stone artefact was of a single broken cobble (K. Kiernan, *Aust. Archaeol.* 15, 85–91; 1982).

In the Franklin Valley itself, there is not only Site F34 Kutikina cave with its rich interleaved hearths containing thousands of stone tools, burnt animal bones, bone points and ochre from between 15 kyr and 20 kyr ago (Kiernan *et al.* *Nature* 301, 28–32; 1983, Ranson *et al.* *Aust. nat. Hist.* 21, 83–87; 1983) but also one open site and 12 cave sites containing stone tools. One of these caves, F66, Deena Reena, is the

largest ever found in the Gordon-Franklin karst, and contains in an erosion gully a geomorphic and archaeological sequence which closely parallels that of Kutikina (Fraser) (Jones *et al.* *Aust. Archaeol.* 16, 57–70; 1983, Blain *et al.* *Aust. Archaeol.* 16, 71–83; 1983). A radiometric programme has not yet been completed, but available assays indicate that this cave, together with one other (F82-6) and the open site (FFS-82) have archaeological deposits dating to the height of the last ice age. By contrast, intensive search by our team on two expeditions into the limestone cliffs of the Gordon valley failed to duplicate this richness, with only one small cave containing a few flakelets in over a cubic metre of excavated deposit and one open site dated to approximately 300 years ago being found.

Dr Paterson also states that there are more than 1,000 known caves in the various limestone regions and in the “coastal and midland sandstone formations” of Tasmania. While only some of these have been inspected by archaeologists, I and many colleagues have over the past 20 years carried out systematic searches of several of these areas. With one exception, dated to 10 kyr and published this year (H. Lourandos *Aust. Archaeol.* 16, 39–47; 1983), no sandstone cave or rock shelter so far investigated has given any evidence of occupation older than 6 kyr. No occupation remains have been found in the extensive limestone caves of northern Tasmania at Mole Creek, nor in the north west Montague. Cave sites on the coast have, with one exception, deposits exclusively restricted to Holocene times.

Before the Franklin finds, the only other proven Pleistocene archaeological sites in Tasmania were Beginner's Luck cave in the Florentine Valley, where some 20 stone tools were found in a geologically reworked deposit dated to approximately 20,000, and Cave Bay cave on Hunter Island where a few stone and bone tools were found in a deposit dated to between 18 and 23 kyr. There was an almost complete hiatus until early Holocene sea-shore occupation times (see S. Bowdler *Adv. World Archaeol.* 1, 39; 1983). The Franklin evidence was to transform this situation.

Yet the original multidisciplinary environmental impact study commissioned by the Hydro-Electric Commission concluded baldly not only that “nothing of archaeological significance has yet been found in any of the caves” but also that “there are no known archaeological sites in the project area”. This so concerned the Australian Archaeological Association that in May 1980 it urged on the Premier of Tasmania that “assessments by professional archaeologists are . . . essential”. Because of their omission, the archaeological significance of the Franklin Valley

was not discovered until the final political decisions about the scheme were being publicly debated. Matters seem now to have improved and environmental impact studies under way in areas of proposed HEC dams on the Henty and Anthony rivers of Western Tasmania include an archaeological component directed by professional consultant archaeologists.

Dr Paterson suggested that archaeologists including myself had entered that “wide gap between truth and verisimilitude when science is subordinated to promoting a cause”. It is of interest to note that the defendants in the High Court case, which included the Hydro-Electric Commission of Tasmania, argued that the archaeological sites of the Franklin “are not significant in the sense that they are important or the only example of such sites”, and further that the number of archaeological remains “in the Kutikina Cave is neither exceptional nor significant”. Now that Dr Paterson claims that the five new caves have the “same” archaeological significance as those on the Franklin River, did he never subscribe to the HEC view, has he changed his mind or does he mean the new sites he has found are also unimportant?

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EMBL's programme

SIR — I would like to correct the impression (*Nature* 17 November 1983, p.214) that within the new research activities at the European Molecular Biology Laboratory, 32 people work under my guidance on haematopoietic cell differentiation. The fact is that less than half of the staff in the new Differentiation Programme work in this area (under the guidance of H. Beug and myself, formerly Krebsforschungszentrum, Heidelberg). Members of the other three groups work on functions of *v-onc* and *c-onc* genes in various systems (R. Müller, formerly Salk Institute, San Diego; B. Vennström, formerly Biomedical Centre, Uppsala) and on aspects of mouse embryo development (E. Wagner, formerly Fox Chase Cancer Center, Philadelphia).

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Gripes

SIR — I would like to add to your list of gripes (*Nature* 10 November 1983, p.134) the use of such terms as “we are at present on a learning curve” (to describe any monotonic time series with negative regression) by writers who obviously are not.

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Just how objective is science?

Norris S. Hetherington*

The supposed objectivity of science has come into question. Recent historical studies reveal instances in which scientific knowledge has not been strictly controlled by observation statements in turn established beyond reasonable doubt by rigorous scientific method. The scientific method has not always proven adequate; scientific observations have, at times, reflected personal biases.

WRITING some thirty years ago, Hermann Bondi suggested that in astronomy, observations had proven a less reliable arbiter of hypotheses than had theoretical considerations¹. Subsequent historical studies have widened and deepened knowledge of some of the instances cited by Bondi and have uncovered additional cases in which supposedly objective facts were subordinated to subjective beliefs.

Many of the transgressions occurred in earlier times, when scientific procedures were less well defined. Added to them, however, are three twentieth-century cases from the world's best observatory. And, were one to go beyond astronomy, there are innumerable cases in which different groups of physicists measuring the same parameter have reported results differing by more than the limits of error. There are even a few instances in twentieth-century physics in which foreseen, anticipated, even desired results were found by scientists adhering to generally accepted scientific methods — even though we now know that the reported phenomena do not exist^{2,3}.

Early astronomical cases

A particular observational result was expected and eagerly sought following the prediction from the Copernican system of a stellar parallax⁴. Robert Hooke was one of the first to use a telescope to search for the phenomenon. Spurred on by the Royal Society, he soon claimed success. There has been a tendency to explain away Hooke's error as actually an observation of stellar aberration. But James Bradley, who discovered and correctly interpreted stellar aberration while repeating Hooke's work, found Hooke's observations "really very far from being either exact or agreeable to the phenomena", that is, agreeable to stellar aberration, and Bradley was greatly at "loss to account for it"⁵. It seems that Hooke found what he expected to find.

Another English astronomer of the seventeenth century open to this charge is John Flamsteed. His parallax measurement agrees well with Bradley's stellar aberration, and astronomers have seized

on stellar aberration as an explanation fitting the facts and not involving personal bias. Flamsteed's understanding of stellar parallax, however, was slightly confused, and his incorrect theory predicted that the pole star would be closer to the pole in winter months than in summer — precisely what he claimed to have observed. Upon becoming aware of his error (it was pointed out to him in private correspondence), Flamsteed blamed his observations on an instrumental error, an error he never succeeded in identifying. The observations are a clear example of a scientist finding what he expected to find⁶.

Astronomers have had equal difficulty in seeing what they did not know to exist. Another English astronomer, Thomas Harriot, drew a very poor representation of the lunar surface after viewing the Moon through a telescope in 1609. After reading Galileo's description of the Moon's surface, Harriot was able to produce a much more detailed drawing. His second map bears a closer resemblance to Galileo's map of the Moon than to the surface of the Moon⁷ (see next page).

William Herschel's observations of the planet Uranus present a striking example of expectation influencing observation. On the evening of 13 March 1781 Herschel discovered what he took to be a comet, newly visible because of its approach near the Earth and Sun. Approaching the Earth, the comet would be increasing in apparent size; that is exactly what Herschel reported, based on a series of measures from 17 March through 18 April. We now know that Herschel was measuring the apparent size of Uranus, at a time when Uranus was moving away from the Earth⁸!

The next planet to be discovered also furnishes an instance of the conversion of expectation to purported fact. The English amateur astronomer William Lassell reported in 1846 his qualified impression of a ring around Neptune, and James Challis, the director of the Cambridge Observatory, soon duplicated the dubious feat. A third observer pointed out in private correspondence that Lassell and Challis were not in agreement on the inclination of the supposed ring, but no one commented publicly on the discrepancy⁹.

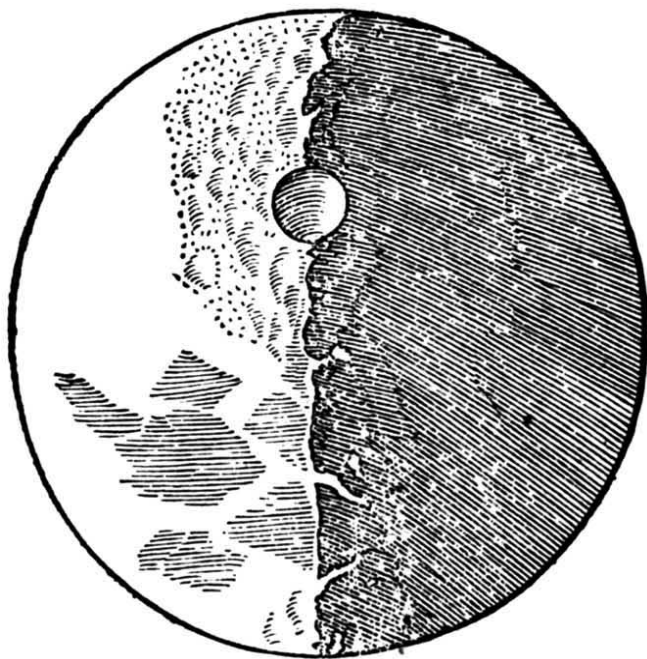


Spiral nebulae

One of three twentieth-century cases from the Mount Wilson Observatory of an astronomer finding what he expected to find, even though it isn't there to find, involves Adriaan van Maanen and the purported photographic detection of internal motions in spiral nebulae¹⁰⁻¹⁵, commonly interpreted as rotation of the nebulae. That the nebulae rotate, though not necessarily rapidly enough to detect photographically, was suggested by their appearance in the nineteenth century and was observed spectroscopically early in the twentieth century. Two years later van Maanen reported his "success" in measuring the rotation from a comparison of photographic plates taken at different times.

His results were readily accepted, because they agreed with spectroscopic evidence and because they were congenial to some theoretical considerations. The plausibility of the measurements was further increased by similar reports from other observatories and by further work by van Maanen. He obtained data for several more spiral nebulae, he had another

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Drawings of the Moon by Galileo (left) and Harriot. They have three idiosyncratic features in common: a large round crater near the centre of the terminator; three arms extending from the light to the dark side of the Moon; and a field of small craters and mountains in the light side. (From ref. 7/Ann Ronan Picture Library-E.P. Goldschmidt & Co.)

astronomer duplicate some measurements, and he ruled out the possibility of an instrumental error by observing a globular cluster and finding no rotation in that instance.

There were some discrepancies, but they were not made public. A few astronomers wrote to each other to point out that the direction of the rotation allegedly detected by van Maanen was different from that determined spectroscopically. And at least one astronomer knew that van Maanen's claim of agreement among different observers was so exaggerated as to be improper, but this criticism, while expressed to van Maanen in private, was not made public.

The major cause for scepticism about the purported rotation came from the implications of van Maanen's alleged measurements for the island universe theory. If the measures were real, then the spiral nebulae could not be independent galaxies at immense distances beyond the boundary of our own Galaxy. And the demise of acceptance of van Maanen's rotations followed quickly upon Edwin Hubble's convincing demonstration in 1924–25, using Cepheid variables in spiral nebulae as distance indicators, that the spirals indeed are galaxies at immense distances from us.

There remained the question of the source of van Maanen's error. Hubble tried valiantly and persistently to attribute the erroneous results to a non-personal factor, and he did find evidence of a magnitude error. (The image of a star builds up differently on a photographic plate depending upon its magnitude, and the centre of the image is less distinguishable for a brighter star.) Ultimately, however, no

random error could explain how van Maanen had found the same sense of unwinding for all seven spiral nebulae. Clearly he had read his expectations into his data, however unsatisfactory an explanation this is for the scientific community. Hubble, unable to describe correctly the source of the error and at the same time fulfil the role of a gentleman scientist, not exposing van Maanen to humiliation, remained silent.

Subsequent purported explanations of van Maanen's results as due to some sort of instrumental error represent a lack of intellectual rigour. They also emphasize a felt need by the scientific community to find any explanation other than personal bias.

Solar magnetic field

A second twentieth-century instance of astronomers finding what they expected to find even though it doesn't exist is that of the reported measurement of a large general magnetic field for the Sun^{16,17}.

At high temperatures elements are ionized and a rotating electrically charged disc produces a magnetic field. A sufficiently intense magnetic field will widen and even split spectral lines. George Ellery Hale, the founder and director of the Mount Wilson Observatory, began searching for the Zeeman effect that would reveal the presence of the predicted general solar magnetic field. Measures by van Maanen found the expected Zeeman splitting, and the expected intensity variation, with a stronger magnetic field at higher solar latitudes. Hale followed proper scientific procedure, and the measurer was not told from which solar latitude the plates he was measuring had come. Later Hale had an English

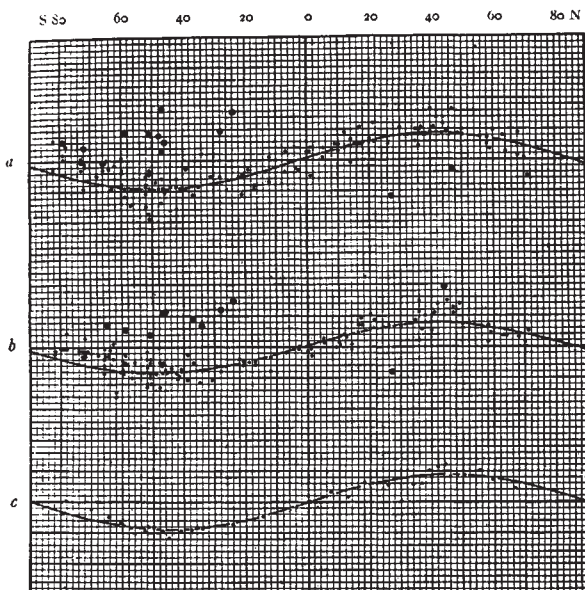
colleague remeasure some of the 1914 plates, and Hale reported that the remeasures confirmed in most cases the earlier results — although in fact the English astronomer had come to the opposite conclusion. Only six years later did the English astronomer make public his contrary results — and then few astronomers believed him. Questions had also begun to arise in the United States, and they also were glossed over. When further observations failed to find a strong solar magnetic field, the initial conclusion was that the field had declined in strength since Hale had measured it.

It was theory, not repeated observation, that exposed the error. Theory came to retrodict a polarity for the Sun's general magnetic field opposite of that reported by Hale for the 1912–16 period. This discrepancy motivated a remeasurement of Hale's plates, a remeasurement which found no significant field at any latitude. "Systematic errors due to some personal bias"¹⁶ were the explanation for the erroneous results.

Sirius B redshift

A third Mount Wilson case, and one not involving van Maanen, is that of the purported measurement of the gravitational redshift of Sirius B¹⁸. The existence of such a redshift, even its amount, was predicted from relativity theory, and Arthur Eddington asked Walter Adams at Mount Wilson to attempt the measurement. That Adams did, finding almost exactly the amount predicted by Eddington.

Eddington was delighted with Adams' purported measurement, but he was also cautious, and wrote that "however exper-



George Ellery Hale's spurious large general solar magnetism field. a And b are for solar lines λ 5812 and 5828; c is a mean curve. It is now known that the magnetic field was not strong enough to measure and its polarity in 1912 was opposite to that reported by Hale. (From Astrophys. J. 44, 210-228; 1913.)

ience the observer, I do not think we ought to put implicit trust in a result which strains his skill to the utmost until it has been verified by others working independently¹⁹.

Apparent confirmation from an independent observer, independent except for the shared expectation of finding a gravitational redshift of nineteen kilometres per second, was soon reported. The currently accepted observed value, however, is approximately four times larger.

There has been an attempt to explain away Adams' results as due to some factor other than personal bias²⁰. His spectra did show some metallic lines, now known not to occur in the spectra of white dwarfs. Obviously Adams' spectra of Sirius B were contaminated by light from Sirius. Adams' results, however, would stand alone on the hydrogen lines in his spectra. Adams' results cannot be explained away as due to contamination from Sirius, however much astronomers would wish to, nor however much astronomers acquiesce in implicit acceptance of the explanation when they do not publicly criticize it.

The inevitability of bias

Recent advances in historical knowledge of cases in which scientists have found what they expected to find even when it wasn't there to find now furnish the basis for a reassessment of the role of observation in science. A common pattern or etiology is now visible.

Clearly there are pressures, internal and external, upon scientists to produce results. A protective mechanism is ignorance of the expected results, and it was in such a mechanism that astronomers at the turn of the century sought protection. The issue of anticipated results is especially evident in the late-nineteenth-century case of Percival Lowell and canals on Mars²¹⁻²³. Lowell had not conformed to the role expected of a scientist, but instead had stated publicly his preconceived opinion that there was intelligent life on Mars. In a

review of Lowell's book *Mars*, W.W. Campbell at the Lick Observatory wrote²⁴ that

Mr. Lowell went direct from the lecture hall to his observatory, and how well his observations established his pre-observational views is told in his book.

Henry Norris Russell at Princeton was slightly more restrained in his 1916 obituary of Lowell, but still managed to argue much the same point that Campbell had: that accepted scientific procedure had no place for preconceived opinion. Russell²⁵ warned that

if the observer knows in advance what to expect . . . his judgment of the facts before his eyes will be warped by his knowledge, no matter how faithfully he may try to clear his mind of all prejudice. The preconceived opinion unconsciously, whether he will or not, influences the very report of his senses, and to secure trustworthy observations, it has been recognized everywhere, and for many years, he must keep himself in ignorance of what he might expect to see.

The problem Russell points to is substantiated by more recent psychological studies²⁶⁻²⁹. The fact that errors in observation are correlated with the expectations of the observers has been demonstrated beyond reasonable dispute. How often measures are biased in the direction of the hypothesis held by the observer is a matter of collective book-keeping.

Scientists have recognized the threat of preconception to objectivity, and in many experiments have followed procedures to circumvent expectation. The use of double-blind procedures in fields such as psychology and medicine is helpful. Observational astronomy poses a special difficulty in that the most interesting observations are often those at the very limits of the instruments available. It is difficult to achieve repeatability of observations, impossible to enjoy the luxury of double-blind measurements.

The disease described by Russell is all too real. Less convincing is the recommended prophylactic: that science should be done (and, by implication, can be done) in the

absence of all preconception. Barring complete lobotomies or other equally effective forms of intellectual castration, it is highly improbable that scientists will not have preconceptions about their world. Baconian science with random observation of all the infinite facts, followed by induction of general laws, and only later the testing of the theories induced, is not a realistic picture of modern science³⁰. Scientists are guided by theory in selecting those observations that may be important, even to the extent of forcing nature to give up information not visible to the casual observer under normal conditions. The creation of theories and their testing, not the random collecting of facts, is a more accurate description of deliberate scientific investigation. The warping of judgment by knowledge, the influence on observational reports of preconceived opinion, is inevitable.

Conspiracy of silence

As bedevilled by preconceptions as scientific observations are, their validity is not to be found in the process of discovery. More promising is the attempt to validate scientific observation through a process of verification after the purported discovery. Such a process depends crucially upon the unrelenting rigour of peer review, however, and historical cases raise serious doubts about the effectiveness of peer review. Peer pressure seems more effective in silencing dissent, or at least so limiting controversy that it does not reach a level where it becomes public knowledge and brings science into public dispute. In some cases the level of controversy is kept so low as to protect the reputations of colleagues even within the scientific community.

At least one astronomer noticed the discrepancy between the Neptunian rings reported by Lassell and Challis, but he notified only Challis. A few more astronomers noticed the discrepancy between the direction of rotation of spiral nebulae reported by Slipher and by van Maanen, but they also severely limited the circle of scientists to whom they reported the discrepancy. Nor were van Maanen's improper claims of corroboration challenged in a public arena.

There is an obvious reluctance to throw the first stone, especially by residents of glass houses. If a young scientist doesn't realize this fact of life, a more senior member of the scientific community can instruct him in the culture of the group. In the case of van Maanen's alleged rotations, Knut Lundmark was initially critical of van Maanen in print. Harlow Shapley at Harvard was quick to inform Lundmark that while he (Shapley) was solely interested in getting at the truth and did not object to just criticism of his own work (somewhat linked to van Maanen's), there was little to gain if astronomers picked to pieces small and irrelevant points, and Lundmark might well think about how many flaws or

hasty conclusions someone might find in his big paper on the distances of globular clusters. Shapley had heard Russell's private criticism of a paper by Lundmark and he was sure that Lundmark would not like to hear it, however much Russell generally admired Lundmark's work¹³. That scientific theories are so sacrosanct that one can determine which anomalies are irrelevant is a disturbing implication of Shapley's letter. The letter was effective, however, in silencing Lundmark.

More difficult to silence was Edwin Hubble, especially after a decade of effort had reinforced his disdain for van Maanen's work and van Maanen's work remained as the outstanding discrepancy in a picture of the universe based largely on Hubble's work. The Mount Wilson bureaucracy did not welcome a public dispute between two of its members. As Frederick Seares, then editor of papers written by Mount Wilson astronomers, explained to Hale³¹,

For two men in the same institution there is opportunity for personal contact and for direct examination of each others' results, and hence for the private adjustment of differences in opinion. The institution itself, it seems to me, is under obligation to see that all adjustment possible is made in advance of publication. If agreement cannot be obtained it may be necessary for the institution to specify how the results shall be presented to the public. . . unless the material presented conforms to standards of dignity as well as of excellence, its acceptance may give the impression of ineffectual direction and cause disruption of morale within the institution itself.

Hubble was not to be completely silenced, but his eventual public statement³² was greatly muted relative to his many unpublished manuscripts on this topic.

There are also pressures on historians of recent science not to be too critical. There has been talk by a few astronomers of closing archives to historians because their initial studies came to unpleasant conclusions. When results have been critical, some astronomers have taken the studies almost as a personal attack, and have responded with questions of the author's competence as a historian (typically in private, not in public).

Another obstacle to effective peer review occurs when observational data is regarded as the private property of the scientist. Such was the case with van Maanen's work on the rotation of spiral nebulae, and Hubble's investigation of the matter was slowed by the necessity to take new plates. Hubble allowed other astronomers free access to his plates.

Peer review could be more effective were data more freely available. It would also be more effective in a climate of opinion that encouraged vigilance by colleagues and competitors. A changing temper in the scientific community, with reputation coming to count for less, is a promising sign

for the progress of science.

Scramble for explanations

A common feature of two of the three Mount Wilson cases, and of earlier cases too, has been the unsuccessful scramble, following the denouement, to explain away the spurious result as an instrumental error. The noticeable exception is that of the spurious general solar magnetic field, and in that case the investigator was a foreign scientist, neither a current nor former member of the Mount Wilson Observatory staff.

In the case of van Maanen's purported rotation of spiral nebulae, Hubble had tried valiantly to attribute the erroneous results to a magnitude error, but was prevented from doing so by the fact that van Maanen had found all seven spiral nebulae that he had measured to be unwinding, while random errors should have produced some unwinding and some winding up¹². Walter Baade, who had worked with Hubble at Mount Wilson analysing van Maanen's work and certainly understood the fundamental obstacle to attributing van Maanen's results to an instrumental error, nevertheless later attempted to explain away van Maanen's error as a magnitude error rather than an error founded upon personal bias³³. Baade's posthumous book may not represent his views fairly; there is less excuse for a more recent statement attributing van Maanen's results to an instrumental error³⁴.

The fact that all seven spiral nebulae were found to be unwinding necessitates the conclusion of personal bias. Yet this conclusion was so unpalatable to astronomers that a recent doctoral dissertation¹⁰ was largely devoted to the attempt to find some other source of error, even though the logic of the situation doomed the attempt from the outset. To the credit of the graduate student, he eventually accepted the logic of the situation and confessed

defeat in his attempt to find a more "reasonable" explanation than personal bias for van Maanen's alleged results.

The case of the gravitational redshift of Sirius B also was exposed by later research at the Mount Wilson Observatory (nearly half a century later). Again there followed an attempt to account for the earlier error as anything other than the result of personal bias. And again the astronomical community silently acquiesced in an obviously inadequate explanation.

The authority of science

Modern science is a remarkable intellectual discipline, its fundamental methods and data generally held to be independent of other facets of our civilization. Its methods are emulated by other disciplines seeking respectability. For many people, modern science provides the ultimate value against which the values of other disciplines are to be judged, much as theology in an earlier age was autonomous, based upon fundamental principles and providing people with knowledge considered to be of ultimate value³⁵.

One threat to the authority of science is the occurrence of outright fraud³⁶. None of the three Mount Wilson cases, however, seem to be instances of conscious fraud, nor are many, if any, of the earlier instances in which scientific objectivity failed. Another threat to the authority of science is posed by the demonstrable influence of subjective elements into what has been widely supposed to be an objective and autonomous enterprise. The decline of theology followed from historical studies revealing that its supposedly divine sources were not absolute, but historically relative, subject to cultural forces. It remains to be seen whether historical studies of science may contribute to a strengthening of science's better features or to a weakening of confidence in modern science. □

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New gene provokes questions

A group at Imperial College, London, has characterized a set of genes which promise to be important for the understanding of normal development and the process that leads to cancer.

THE article on page 756 from Dr P. W. J. Rigby's group at Imperial College is an intriguing document which is plainly of the utmost importance, but for reasons which, at this stage, are not fully apparent. The obvious difficulty is that the observations described bear on such a variety of important but unanswered questions. What Rigby and his associates describe, in this and two closely related articles (Scott *et al.* *Cell* **34**, 557; 1983 and Murphy *et al.* *Cell*, in press), is the characterization of sets of genes, silent in most normal mouse cells but active in cells that have been transformed, or given the properties of malignancy, by the cancer-causing DNA virus SV40. So is the activity of the genes concerned characteristic of the cancerous condition? That is an obvious and important question, and for at least one set the answer must be affirmative, since the same or similar genes have been found to be activated in malignant cell lines originally derived from a variety of mouse cell types and transformed by various carcinogens, chemicals as well as viruses. This is by no means the first time a possible genetic marker has been identified in tumour cells. But what makes this discovery particularly important is that — taken with other evidence on the activation of the same genes in normal embryos — it suggests for the first time how the normal mechanism of cell differentiation may be disrupted in tumour cells.

If this were the only importance of the new development, nobody would complain. The excitement of the new development is its ramifications into other quite unexpected fields. One is the recognition that the prototype of one set of genes appears to be virtually identical to genes encoding the histocompatibility or transplantation antigens. The most familiar histocompatibility genes are those responsible for the idiosyncratic membrane proteins known as the transplantation antigens (H-2 in mice and HL-A in people), which enable the immune system to tell the difference between self and non-self. The newly characterized gene belongs to a distinct subset of these genes, closely linked to those encoding the more familiar histocompatibility genes, at what is called the *Qa/Tla* locus.

The importance of this development for the understanding of the immune system, while far from clear, seems certain to be considerable. Elizabeth Simpson gives a brief account of what this may be on page 738. Echoing Brickell *et al.*, she suggests

that the genes in question may normally be active only in the earliest stages of the development of an embryo, before the maturation of the immune system, so that their products would not be recognized as "self" proteins if they should appear at some later stage. It is fascinating that this should tie in with Medawar's observation more than a decade ago that animals can be partially protected against later exposure to carcinogens by immunization with embryonic tissues.

This is not the end of the tale. The phenomena now described from Rigby's laboratory seem to be linked with the broad general question of the mechanism whereby the cells of different tissues acquire their particular properties. It has long been suspected that the apparently functionless repetitive sequences of nucleotides that are widely distributed in the chromosomes may in fact play a part in coordinating the differential activity of genes in different tissues. It is therefore notable that one of the sets of genes activated in tumour cells has now been found to share a repetitive sequence with a number of genes actively transcribed in the tissues of mouse embryos at a specific stage of development (Murphy, D. *et al.*, *Cell* December 1983, in press). This may provide for the first time a direct way of attacking the puzzling problem of differentiation.

The techniques used to isolate these sets of activated genes are of some interest in their own right. The problem faced by Rigby and his colleagues was that of detecting the activity of genes that, even in the tumour cells, were active only at relatively low levels. Their solution to this problem is, in principle, simple. In effect, they subtracted copies of all of the genes active in the normal parent cell lines from copies of the genes active in the transformed (cancerous) cell lines. What they were left with were discrete sets of genes active only in the tumour cells.

These are the techniques also being used to good effect to identify, for example, the genes responsible for antigen recognition in T lymphocytes. The problem in both cases is, so to speak, to find a needle in a haystack. The task facing Rigby's group is complicated by the huge number of needles there seems to be. There is a formidable programme of work lying ahead.

Where will it lead? For the past few years, the most immediate task in molecular biology has been to understand

the regulation of genes in organisms more complicated than bacteria. It is generally assumed that this depends on the binding of regulatory proteins to specific DNA sequences flanking the functional part of genes. One suggestion for how oncogenes disrupt normal cellular behaviour is that they mimic such regulatory proteins. In the light of Rigby's results, it now seems possible that one of the products of the SV40 virus (the large T antigen) may be just such a mimic and that it may have cellular homologues normally active only at specific stages of development. A candidate for one of these homologues is the cellular *myc* gene, whose activation in lymphoid tumours is discussed by Miranda Robertson on page 733. Both the large T antigen and the *myc* product, as well as the product of a second viral oncogene (the E1A gene of adenovirus) are known, as she discusses, to be functionally equivalent in that they can confer on cells in culture the ability to proliferate indefinitely. Furthermore, there is evidence suggesting that the products of *myc* and E1A are DNA-binding proteins that are capable of activating genes that would normally be silent. Precisely what DNA sequences are bound by the E1A and *myc* products is not known, but in the case of the SV40 T antigen, the binding sites on the viral DNA have been very well characterized. And perhaps the most exciting of Rigby's recent discoveries is preliminary evidence that those binding sites have sequences in common with the repetitive element distinguishing one of the sets of genes activated in transformed cells.

This evidence that repetitive sequences — in this case relatively short ones — may be important in the coordinate regulation of groups of genes during development seems to vindicate a notion promulgated over some years by Roy Britten and Eric Davidson (see *Nature* **301**, 468; 1983). Their thesis has been precisely that small repetitive elements flanking functional genes may participate in the coordinate regulation of groups of genes during embryonic development. As an extension of that idea, they have pointed out that these small repetitive elements seem to be capable of changing their positions in the chromosomes during evolution; this, Britten and Davidson have suggested, may bring about the sort of changes in developmental regulation that have led to the evolution of new structures from old ones. □

Ecology

The birds of Selborne

from John H. Lawton and Robert M. May

GILBERT White's *The Natural History of Selborne* is arguably the first published work on ecology. The book is cast mainly in the form of letters from White — who lived in Selborne, Hampshire for most of his life (1720–1793) — to two friends. It differs from earlier works on descriptive natural history in that it sees individual plants and animals not as things by themselves, but rather as parts of a community of living organisms, interacting with the environment, with other organisms, and with man. Since it appeared in 1789, the book has exerted a continuing charm, running steadily through more than 200 editions and translations. Indeed, if a recent edition¹ is to be believed, it is now the fourth most published book in the English language.

White's blend of detailed observations about natural history and concern for basic ideas is well seen in the following passage, written in 1778¹:

Among the many singularities attending those amusing birds, the swifts, I am now confirmed in the opinion that we have every year the same number of pairs invariably; at least, the result of my inquiry has been exactly the same for a long time past. The swallows and martins are so numerous, and so widely distributed over the village, that it is hardly possible to recount them; while the swifts, though they do not all build in the church, yet so frequently haunt it, and play and rendezvous round it, that they are easily enumerated. The number that I constantly find are eight pairs, about half of which reside in the church, and the rest in some of the lowest and meanest thatched cottages. Now, as these eight pairs — allowance being made for accidents — breed yearly eight pairs more, what becomes annually of this increase? and what determines every spring, which pairs shall visit us, and re-occupy their ancient haunts?

The passage is remarkable for its clear exposition of the central question of population biology: what regulates populations? It is also unusual in presenting quantitative information about the population of swifts in Selborne two centuries ago, a small exception to the almost general absence of population records that go back more than a few decades. With this in mind, we travelled to Selborne in July to see how many swifts are found there today.

Several changes in the physical setting should be noted. Comparisons of old and new maps show that the patterns of field and woodland around Selborne have changed little over the past 200 years. But, as emphasized by Selborne's current verger and naturalist, Stephen Povey, the woodland is less coppiced and otherwise managed for sustained yield of firewood and other products, so that undergrowth is thicker than in White's time. These changes, and other parallel changes in the

management of streams and pastures, could result in changes, probably increases, in the abundance of the insects that are the swifts' food supply. The church tower, in which roughly half of White's swifts nested, was rebuilt in 1781. More significantly (as explained by William Andrews, the church warden), the tower windows were stopped up with wire shortly after World War II to keep out the grey squirrels that then infested it. As the squirrels had been in residence for several years before their removal, it is unlikely that swifts have nested in the tower for some 50 years. As for the other half of White's swift population, the 'lowest and meanest thatched cottages' are now gone, or reroofed with material less congenial to swifts, or gentrified with wire wrapping the thatch.

Despite these changes, Stephen Povey's observations are that around twelve breeding pairs of swifts are regularly to be found in Selborne in the summer. In view of the many unquantified changes that have taken place in the intervening two centuries, we regard this number as strikingly close to the eight pairs so consistently found by Gilbert White. Were it not for its uncontrolled and anecdotal character, this could be claimed to be the longest running field study in ecology.

The other population patterns that White described in the above paragraph also persist to this day. Thus the breeding pairs of swifts each fledge roughly two offspring each year, to give a total population of about 50 swifts at the end of the summer. The house martins continue to be more numerous than the swifts, with population sizes ranging around 150 to 200 in recent years; their abundance is significantly more variable than the swifts'. Likewise, the population of swallows remains more numerous than that of swifts, being intermediate in magnitude between the swifts and the martins.

All this leaves the central question unanswered: what ultimately keeps the population of swifts relatively constant? Many naturalists would be inclined to say that availability of nesting sites is the determining factor, but the slight increase in the swift population of Selborne over the past two hundred years, in the teeth of the disappearance of most of their eighteenth-century nesting places, casts some doubt on this. The regulating factor could nevertheless be some combination of food supply and nesting sites, or the explanation could even lie in their wintering grounds to the south (in White's day, it was conjectured that swifts hibernated, somewhere locally, for the winter).

The dull brown swifts are not among the

gaudy species adorning a commemorative window in Selborne church (see the cover of this issue of *Nature*). As with aerial-feeding martins and swifts, the relative abundances of members of other guilds represented on the window have probably changed little since the eighteenth century. Bullfinches and crossbills — both seed-feeding finches — are, for example, still much scarcer than chaffinches, both locally within the parish of Selborne, and nationally. They illustrate an intriguing but poorly understood relationship between local population dynamics and geographic distribution. Typically in many taxa (not just birds), rare species (nightjars are a good example) occur at rather few sites and tend never to be common when they do occur. The reverse is true for common species^{2,3}.

One theoretical explanation for these patterns, first put forward by I. Hanski⁴⁻⁶, requires common, widespread species and rare, local species to reverse status over time, in a haphazard and unpredictable manner, even in a constant environment. As we have tried to show, this is not what usually happens. Of course, some of the birds of Selborne have changed status since White's day, either increasing (for example, the siskin) or disappearing entirely from the parish (for example, the raven and woodlark). Such changes are more likely due to changes in habitat or persecution, than to the processes of stochastic colonization and extinction envisaged by Hanski.

Populations of several of the species pictured on the window have now been well studied by White's spiritual descendants — that army of dedicated amateur ornithologists for which Britain is rightly famous (or viewed with mild disbelief, depending upon whether or not you are a bird watcher). Much of their work is nurtured and coordinated by the British Trust for Ornithology (BTO). The BTO data on herons, for example, span 56 years from 1928, making them the longest running continuous series for any European breeding bird⁷. They show a well regulated population, perturbed only by severe winters, but otherwise stable at between four and five thousand pairs. As is the case with swifts, the mechanisms of regulation are poorly understood.

The BTO's Common Bird Census has a shorter pedigree, documenting patterns of population behaviour for most of Britain's commonest breeding species (wren, skylark, robin etc.) since 1962. A good example of the potential usefulness of these data is Pimm's attempt⁸ to employ them in a tricky problem of parameter interpretation for stochastic population models.

Obviously, answers to questions about the relative abundance of species demand data on rare, as well as common, species. The brightly coloured hoopoe and woodchat shrikes on the church window were as much a novelty for White as they are for modern British birdwatchers. (He

thought hoopoes 'the most unusual birds I ever observed in these parts'.) A few hoopoes stray to Britain from southern Europe every year, but rarely stay to breed. Hence views on persistence and stability of populations (see, for example, ref. 9) must inevitably be coloured by where ecologists work and what they study. Those who study rare species, particularly populations at the edges of their range, may be impressed by instability of numbers and high frequencies of extinction. Things may be quite different at the centre of a species' distribution. Unfortunately, we remain remarkably ignorant about what happens at the centre compared with the edge of a species' range¹⁰.

In sum, the birds that grace the Selborne window and the marvellous swifts thought too dowdy to join them, typify much of contemporary population biology. Facts about population magnitudes are often well documented, and there is no shortage

of interesting questions. Unfortunately, the mechanisms that produce steadiness in populations, or cyclic changes, or irregular fluctuations — that is the answers to the questions — are much debated and still rather poorly understood. □

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Oncogenesis

Paradox and paradigm: the message and meaning of *myc*

from Miranda Robertson

THE cellular *myc* gene may, depending on your point of view, be seen as either the most illuminating or the most confusing of the cellular oncogenes. The promise of illumination lies in the overwhelming circumstantial evidence that *c-myc* activation in some lymphoid tumours is a direct subversion, through chromosomal translocation, of the mechanisms by which immunoglobulin genes are activated in normal lymphoid cells. Yet the more becomes known about both immunoglobulin gene expression in normal cells, and *c-myc* expression in abnormal ones, the less clear it is how the two fit together. A series of recent papers on human lymphomas, three of them in this week's *Nature*¹⁻⁵, illustrates how they defy unified explanation. At the same time, however, a subtle shift in the emphasis of research — discernible at a meeting last month in Berlin* — has brought into focus consistencies in *myc* behaviour that should help to illuminate the mechanism of tumorigenesis not only in B lymphomas (which are relatively rare) but in other kinds of cancer as well. Until now, arguments have tended to polarize on the issue of whether quantitative changes in the oncogene product are sufficient (or even necessary) for tumorigenesis, or whether there must be some structural change. The B lymphomas obligingly provide some support for both points of view. But what they consistently reveal is an aberration in the regulation of the *c-myc*

oncogene specifically in the context of normal lymphoid differentiation, in which it plays at some stages a perfectly legitimate part. Such aberrant interactions between oncogenes and the normal differentiation programme of cells may explain much of the tissue specificity of at least some of the viral and cellular oncogenes, as well (possibly) as some of the phenomena of tumour evolution.

The B cell background

The mechanisms of immunoglobulin regulation and their implications for *c-myc* activation have already been touched on this year in *Nature*^{6,7} and concisely and judiciously reviewed elsewhere by Perry⁸. I shall briefly recapitulate the main events in the differentiation of antibody-secreting B lymphocytes before discussing the most recent data on B lymphomas and their implications for tumorigenesis in general.

The first step in B-cell differentiation is a genomic rearrangement of the immunoglobulin gene complex that brings one of a large number of transcriptionally silent variable-region gene segments (*V* segments) into the transcriptionally active site of a constant-region gene segment (*C* segment). (The organization of immunoglobulin gene segments is outlined, for non-cognoscenti, in Figure 1.) A promoter upstream of the *V* segment is thus brought under the influence of an enhancer sequence in the intron upstream of the *C* segment, with a net increase in the transcriptional activity at the site and the production of a complete immunoglobulin transcript. A crucial point for the later discussion of *c-myc* activation is that this

transcriptional enhancement is strictly confined to the rearranged *V* segment: the upstream *V* segments, despite having functional promoters, remain silent.

Once the immunoglobulin genes have undergone the rearrangements necessary to encode a complete molecule, immunoglobulin is expressed on the surface of the cell where it can bind antigen and signal the next step in differentiation, which involves a switch from surface to secreted immunoglobulin and a further increase in transcriptional activity.

What happens in about 85 per cent of mouse plasmacytomas and some 50 per cent of human Burkitt's lymphomas is a chromosomal translocation that brings the *c-myc* oncogene, which is not normally transcribed in these fully differentiated plasma cells, into the highly transcriptionally active immunoglobulin locus where it is transcribed at levels varying over a five-fold range. The obvious inference at first sight is that the *c-myc* genes in these tumours are activated, like the immunoglobulin genes in normal cells, by the combined effect of the *V*-segment promoter and the enhancer associated with the *C*-segment. Any contribution of the *V*-segment promoter was however ruled out early on by the discovery that the translocated *c-myc* gene is in the opposite transcriptional orientation to the immunoglobulin locus, 'head-to-head' (see Figure 2). Four different groups have now examined four different tumour cell lines in search of alternative explanations for *c-myc* activation, and have arrived at four different conclusions.

Translocations in tumours

Most of the results so far come from translocations between chromosome 8, which contains the *c-myc* locus, and chromosome 14, which contains the immunoglobulin heavy-chain locus; a few are beginning to emerge from much rarer translocations (so-called variant translocations) involving the light-chain loci on chromosomes 2 and 22.

The most obvious solution to the orientation problem lies in the immunoglobulin enhancer element recently discovered in mouse^{9,11} and now described in man by Rabbitts *et al.*², Mills *et al.*¹² and Hayday *et al.*⁵ in this and forthcoming issues of *Nature*. The enhancer is tissue specific, but (by definition) indifferent to gene orientation and could thus account in principle for the transcriptional activation of the front-to-back translocated *myc* gene. Indeed Hayday *et al.* have succeeded in confirming that in Manca B lymphoma cells *myc* transcription is dependent on the immunoglobulin enhancer. Alas, this neat solution is so far applicable only to Manca cells. Battey *et al.*¹ have looked at BL22 cells, ar-Rushdie *et al.* at JD38 and ST486 cells¹³, Gelmann *et al.* at ST486 cells⁴ and Rabbitts *et al.*² at Raji cells, all derived from Burkitt's lymphomas, and report that in these cell lines the translocation moves the immunoglobulin enhancer to chromosome

*A Dahlem Conference on Leukaemia was held in Berlin on November 14-18. The proceedings will be available as a book which will be published by Springer-Verlag in summer 1984.

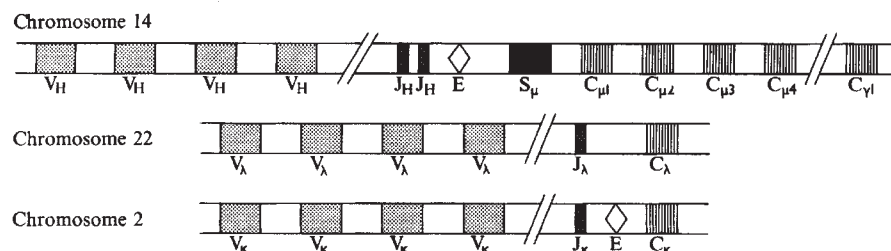


Fig. 1 Immunoglobulin molecules are composed of two light chains and two heavy chains each. The heavy chains are encoded on chromosome 14 of man. The light chains exist in two isotypes: λ , which is encoded on chromosome 22, and κ , which is encoded on chromosome 2. The genes for all three types of chain are assembled in the course of B-lymphocyte differentiation by a chromosomal rearrangement which brings one of a large number of variable-region segments (*V* segments) into juxtaposition with one of a smaller number of *J* segments adjacent to the constant-region genes (*C* segments). (In the case of the heavy-chain genes a third variable segment, *D*, intervenes between the *V* and *J* segments: this has been omitted for the sake of simplicity.)

In the case of the heavy-chain genes, the assembled variable-region segment undergoes further rearrangements later in B-lymphocyte ontogeny. These rearrangements involve a switch in the constant-region gene expressed with a given *V* segment, and are mediated by specialized switch regions upstream of each of the five constant-region genes. The first constant region to be expressed is μ ; later most B lymphocytes switch to γ . E, enhancer; S, switch sequence.

8 where it cannot influence the translocated oncogene on chromosome 14.

Since in all these cell lines the translocated *myc* sequences are nonetheless expressed, this implies that the *V*-segment promoters and the C-segment enhancer may not provide the full explanation for immunoglobulin gene expression and certainly do not explain the expression of the translocated *c-myc*. This may not be entirely surprising since a third mechanism is in any case required to explain the increase in transcription that accompanies the production of secreted immunoglobulin; and moreover recent evidence suggests that there is no enhancer sequence associated with the lambda light-chain immunoglobulin locus¹⁴.

There are in any case many who do not believe the explanation for tumorigenesis is to be found in the simple transcriptional activation of oncogenes in the absence of structural changes in the gene, and have looked to the properties of the translocated *c-myc* rather than those of the immunoglobulin locus for the mechanisms of activation. They were greatly encouraged by evidence, originally from mouse plasmacytomas, that the cellular *myc* gene is broken in the translocation¹⁵, and particularly when it later emerged that the breakage results in the loss of an entire 5' exon, present in the normal cellular gene but absent from the viral *myc* sequence which corresponds only to exons 2 and 3 of the cellular gene (see Fig. 2a). This apparently highly significant discovery is however complicated by the fact that the 5' exon contains three in-phase stop codons and is plainly not translatable^{16,17}; the intact cellular *myc* gene, the truncated gene (which is transcribed from a number of cryptic promoters in the first intron⁵) and the viral gene thus all produce a protein corresponding to the last two *myc* exons.

But many have found it hard to accept

that this means the loss of the first exon has no significance for tumorigenesis. Allowing that qualitative changes seem to be ruled out, they have turned back to the possibility of quantitative changes and suggest that exon 1 may have a crucial effect on either transcriptional¹⁸ or translational^{1,16} control. In particular, Leder¹⁸ has postulated a transcriptional repressor acting in *trans* on the exon 1 sequence. It is thus something of an embarrassment that the *c-myc* gene is not in fact truncated in BL2 or Raji cells, or in a third Burkitt's cell line, Daudi¹⁹. In the hope of identifying alterations that might be critical to tumorigenesis, workers in Leder's and Rabbitts's laboratories have now sequenced the translocated *c-myc* genes of GL22 and Raji respectively, and examined their patterns of transcription^{1,3,20}.

Both find *myc* transcripts of two different sizes, 2.2 kb and 2.4 kb, attributable to two promoters at the 5' end of exon 1^{1,20,21}. Battey *et al.* find the 2.4-kb transcript relatively over-represented in BL22 by comparison with normal lymphoblastoid cells, which express modest levels of *c-myc*. They point out that the potential secondary structures of the untranslated mRNA are quite different for the two sizes of transcript, and suggest that the increased use of the upstream promoter in BL22 may help to release the gene from translational control. In support of this idea, they stress the point that the 5' untranslated exon is very highly conserved and cite the case of the alpha 1 amylase gene, which is differentially regulated in salivary gland and liver apparently on the basis of differential splicing of its 5' untranslated leader sequence.

It is not entirely obvious how such a shift in the balance of the two transcripts in tumour cells could have very profound effects on their growth; and furthermore, Hamlyn and Rabbitts²⁰, comparing pat-

terns of *myc* transcription in several tumour cell lines, do not find a consistent predominance of the 2.4-kb transcript (though it is true that the 2.2-kb transcript is always relatively depressed). Given the strong conservation of exon 1 the existence of these alternative promoters must mean something, but it is not clear that their differential use plays any part in tumorigenesis. And in the meantime, sequence data from the Raji cell line have enabled Rabbitts *et al.*³ to offer a much more spectacular alternative answer to the question of how the translocated *c-myc* differs from its normal counterpart: what they find is that the 5' half of the translocated gene is littered with mutations, 16 of which produce amino acid substitutions in exon 2 which is also expanded by the insertion of a leucine triplet.

This may, once again, reflect the specialized properties of the immunoglobulin locus. The site occupied by the translocated *myc* gene is the normal home of the *V* segment, whose variability is now known to be increased in the course of B-lymphocyte differentiation by somatic mutation. From this point of view, it may be significant that in Raji cells *myc* is translocated to the site of the γ constant-region gene instead of to the much more usual μ . Normal B cells switch from μ to γ in the later stages of differentiation (see Figure 1), and it is believed that the generation of mutations is associated with the switch.

Indeed, Rabbitts's discovery may in the end prove more significant for B-cell ontogeny than for leukaemogenesis, since he finds no mutations in the translocated *c-myc* of Daudi cells and Battey *et al.* find none in that of BL22 cells. C. Croce (Wistar Institute) similarly reported in Berlin perfect conservation of two translocated *myc* sequences, before going on to summarize findings that confound all current theories of *c-myc* activation, including perhaps (to some extent) his own.

He and his colleagues have from the beginning been associated with the transcriptional activation hypothesis, for which they were able to provide elegant experimental support by hybridizing a Burkitt's cell with a mouse plasmacytoma and showing that daughter cells retaining the intact human chromosome 8 produced no detectable human *myc* transcripts whereas those retaining the human chromosome 14 containing the translocated gene produced high levels²². On the basis of these experiments, Croce and his colleagues have refined the idea of *myc* activation to what he calls *myc* deregulation, and propounded a theory of lymphomagenesis based on the interaction of the translocated *myc* gene with tissue-specific factors dictating differential patterns of gene expression at successive phases of B-cell differentiation.

The cornerstone of his thesis is a series of cell hybridization experiments, including the one described above, showing that the normal *c-myc* gene is regulatable in cells of the B lineage whereas the translocated gene

is not. Thus when human lymphoblastoid cells, which normally produce *myc* transcripts, are fused with mouse plasmacytoma cells, the two normal human *c-myc* genes are silenced²². This implies *trans*-acting suppression of *c-myc* transcription in the plasma cells. The translocated *c-myc* escapes inactivation by invading a region of DNA that becomes constitutively active at the plasma-cell stage, when the normal *c-myc* is shut off. This point has also been clearly stated by Bernard *et al.* in a recent survey of *myc* translocations³⁸.

The nature of the constitutive activation however still remains something of a puzzle. The one generalization that seems possible so far is that it depends on a property of the C-segment locus, acting on upstream sequences. This last conclusion is based on recent analyses of the so-called variant translocations — those involving the light-chain immunoglobulin loci on chromosomes 2 and 22. In both these cases, the *c-myc* gene remains on chromosome 8, and the constant-region genes become joined to its 3' end (see Figure 2b)²³⁻²⁵. Thus the orientation of the gene relative to the immunoglobulin locus does not seem to matter, provided that the oncogene is at the 5' end of the constant-region segment. This of course makes sense, since it is the V-segments 5' to the constant-region locus that must be activated in normal B cells. What does not make sense is the transcriptional activation of the translocated *c-myc* gene in Daudi cells, in which the *myc* gene is translocated intact to a site some 50 kb upstream of the C-segment locus, with several V-segments between the two. All the evidence from normal B cells is that transcriptional activation extends only as far as the first upstream V⁸; so once again it is difficult to see how the activation of the translocated oncogene fits in to the B-cell differentiation programme.

Immortalizing proteins

Yet while the detailed interpretation of *myc* translocations in B lymphomas becomes increasingly difficult, the broad outlines remain remarkably clear. Furthermore, Klein *et al.* have just established that the 6:7 translocations that are a common feature of rat plasmacytomas also involve a *c-myc* and the immunoglobulin locus — thus extending the phenomenon to another species²⁶.

What are the implications of this consistent phenomenon in broader terms? First, it suggests that the tissue specificity of at least some of the oncogenes may lie in their mode of regulation rather than in any specific action of the product. This is consistent with evidence that *c-myc* may be activated in epithelial as well as in lymphoid tumours. It may also explain at least in part the apparent tissue specificity of some oncogenic viruses: for example W. Haseltine (Harvard) reported in Berlin that a murine leukaemia virus that is non-virulent in T cells could be converted into a virulent and tumorigenic one by structural alterations affecting only the long-terminal-repeat

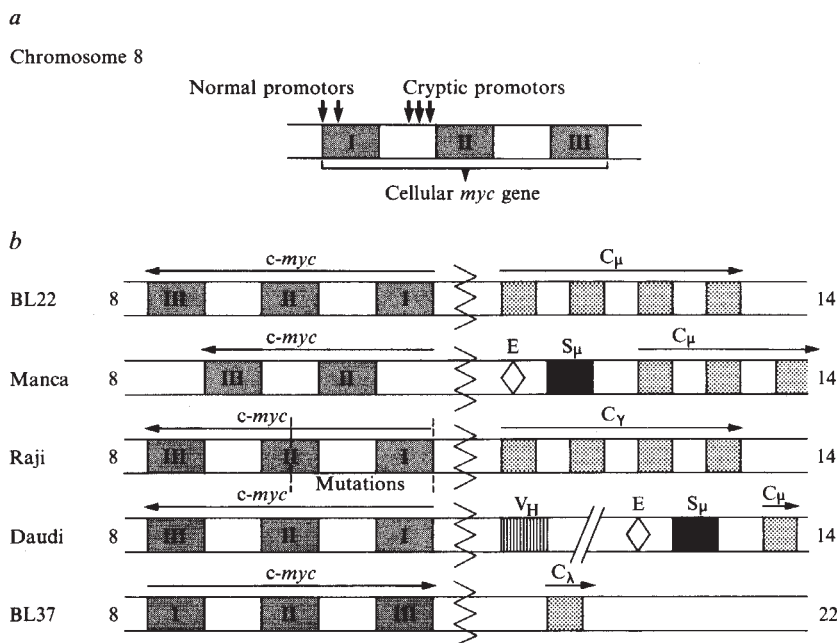


Fig. 2 *a* Cellular *myc* gene. *b* Structure of the *c-myc* gene and immunoglobulin genes at the breakpoint in five Burkitt's lymphoma chromosomal translocations, four being 8:14 translocations involving the immunoglobulin heavy-chain locus and one so-called variant translocation between chromosome 8 and the λ light-chain locus on chromosome 22. Arrows indicate the direction of transcription.

regulatory sequences. At the same time, this means that a mutation or translocation that would activate an oncogene in one tissue would not necessarily activate it in another: thus when Burkitt's cells are hybridized with fibroblasts, the translocated *c-myc* gene is inactivated²², presumably because fibroblasts do not normally transcribe immunoglobulin genes and therefore do not recognize the altered regulatory signals. A further implication of the hybridization experiments is that there are suppressor factors acting in *trans*: thus inactivating mutations affecting suppression could explain tumours that seem to arise through recessive mutations. (Such a case is retinoblastoma²⁷).

Finally, how does *c-myc* activation actually contribute to tumorigenesis? Any answer to this question must at this stage be speculative; but in the light of recent developments the temptation to speculate is hard to resist. First, it is clear that *myc* activation alone is not enough to induce full malignant transformation: G. Klein (Karolinska Institute) pointed out that many people have lymphocytes with typical Burkitt's translocations without ever developing tumours. What the evidence so far suggests is that the contribution of the *c-myc* product is to immortalize the cells, while a second unknown oncogene — and possibly others — contribute other aspects of malignant transformation^{28,38}. More recently, preliminary experiments with *myc* and other immortalizing genes have suggested that this category of oncogene acts by inducing in turn the transcription of other genes that would normally be silent.

According to investigations on both the viral and the cellular *myc* product, it is a

DNA-binding protein^{29,30} found almost exclusively in the nucleus. This distinguishes it from the products of oncogenes such as *ras* and *src*, which seem to be associated with the cell surface. Furthermore, transfection experiments in which combinations of two oncogenes are introduced into tissue culture cells suggest that *myc* supplies a tumorigenic function different from that of *ras*, but essentially similar to the DNA viral transforming genes E1A of adenovirus and large T of polyoma virus^{31,32}, both of which act to immortalize cells without fully transforming them, and both of which in turn affect the regulation of other genes.

Inspired by these apparent functional homologies between E1A and *myc* (which also have some sequence homology³³), several groups have now gone on to do further transfection experiments, briefly mentioned in a recent report in *Nature*³⁴. They have shown that otherwise inactive exogenous genes (for dihydrofolate reductase in one case and β globin in the others³⁵) can be activated in heterologous cells if the cells are co-transfected with E1A; and in the case of the dihydrofolate reductase gene the experiments have been extended to co-transfection with *myc*. Interestingly, only the exogenous and not the endogenous β globin genes are activated by E1A, which suggests again that the differentiated state of the cell — presumably as manifested in its chromatin structure — is important in determining the aberrant activation of its genes.

The evidence that some oncogenes may work by activating genes that are inappropriate to the differentiated state of the cell is moreover supported by evidence of quite a different sort from tumours induced by

chemical mutagens as well as by viruses. These cells express specific identifiable sets of genes that are inactive in the non-transformed parent cells and can be distinguished by characteristic repeated sequences co-transcribed with them^{36,37}. It is beginning to look as though the study of aberrant gene activity in tumour cells *in vitro* may soon lead to a real understanding of gene regulation in both normal and abnormal cells. □

Miranda Robertson is an associate editor of Nature.

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Deep sea drilling project

On the Mississippi Fan

from the staff of Leg 96

LEG 96 of the Deep Sea Drilling Project took the drilling vessel *Glomar Challenger* to the Mississippi Fan where nine sites were cored from September 29 to November 8, 1983. The aims of the visit were to study the distribution of sediment types in the uppermost few hundred metres, to analyse the transport processes responsible for resedimenting shallow water sediments in the deep sea, to study the time framework required to transport such large quantities of material, and to determine the geochemical characteristics of the deposits.

The Mississippi Fan, located in the Gulf of Mexico, is one of the largest submarine fans recognized in our modern oceans. Bathymetry, side-scan sonar, and seismic reflection data show that it has an area of about 290,000 km², a volume of about 300,000 km³ and that it was constructed during the Pleistocene period. Seismic data further indicate that the broad wedge-like accumulation of Mississippi Fan deposits consists of at least seven discrete sediment units, called fan lobes. Comparison of isopach maps of each fan lobe reveals that the slightly convex upward surface of one fan lobe results in an offset of the succeeding one, and that the sediment source of each is located at a different site on the adjacent outer shelf.

Four major physiographic zones can be distinguished on the youngest fan lobe: canyon, upper fan, middle fan, and lower fan. Mississippi Canyon, which is the sediment source for the youngest fan lobe, is located on the outer shelf and upper slope,

about 60 km west of the present Mississippi Delta. It commences at a water depth of 150 m, has an average width of 12 km, and gradually merges with the upper fan at an approximate water depth of 1,200 m. This canyon formed during a period of low sea level in a relatively short period of geologic time, probably as a result of sediment instability on the slope. The upper fan is characterized by one large, nearly filled channel with poorly-developed levees. At approximately 2,000 m water depth, the upper fan changes into an elongate middle fan lobe that is about 150 km wide and nearly 400 m thick; it is convex upward in cross section. The middle fan central channel is about 3 km wide, is highly sinuous, and has broad levees that are about 20–40 m higher than the present channel floor. Downslope, the channel becomes smaller and displays less sinuosity. The lower fan begins at approximately 3,100 m water depth. The channel on the lower fan is generally less than 500 m wide, and disappears near the terminus of the fan lobe. Abandoned channels are found adjacent to the youngest channel, suggesting that channels switch through time rather than migrate laterally.

Of the nine sites drilled on the Mississippi Fan, four were in the lower fan and five were in the middle fan. Seven of the holes were drilled to a depth of about 150–200 m in the youngest fan lobe. Two deeper holes, one in the lower fan (523 m penetration) and one in the middle fan (442 m penetration), were drilled to investigate

the sedimentary characteristics of the underlying fan lobe(s). Core recovery and quality were generally excellent in the upper 100 to 150 m at each site, but decreased considerably as more consolidated clays and loose sands were encountered.

To complement the study of the cores, sonic, gamma ray, and caliper well logs were run at four sites on the middle fan and at three sites on the lower fan. The sonic tool determines the transit time of a compressional wave through the formation adjacent to the tool. The gamma ray tool measures the natural background gamma-ray emissions caused by the decay of potassium, thorium, and uranium salts commonly found in sands and muds, and is therefore a valuable indicator of sediment type. The caliper measures the diameter of the hole and keeps the logging tool more or less centered in the hole which increases the accuracy of the other measurements.

The five sites occupied in the middle fan provided a cross section from the levee, across the channel, onto the overbank region, and out to the lateral margins of the fan lobe. An unexpectedly thick section of gravel and pebbly mudstones was drilled in the channel at a depth of about 200 m. These gravels were overlain by sands that pass upsection into muds with thin sand and silt layers. The upper 50 m consist primarily of homogeneous muds. The well log through these channel fill deposits shows a fining-upward trend. The basal sand and gravel beds vary in thickness from 1 to 5 m, and show sharp bases and rather abrupt tops. Upsection, the fine sand, silt, and mud interbeds vary from 2 to 10 m in thickness, and show an irregular and ragged log response. Core recovery was generally poor in the sandy and silty units, giving a false impression of the overall sand percentage. Logging results shows that much of the non-recovered channel deposits are sands; interpretations based solely on recovered core segments indicate an erroneously low sand percentage.

The site drilled in the middle fan levee clearly documented a fining-upward trend. Basal muds contain thin sand and silt layers and become more homogeneous muds uphole. The silty and muddy sediments were generally well stratified and were deposited by low velocity density currents forming thin-bedded mud turbidites.

The site on the middle fan overbank deposits, located approximately 18 km away from the middle fan channel, was rotary cored with relatively low core recovery. Combined coring and logging results indicate that the overbank sediments are composed primarily of fine-grained sediments arranged in alternating coarsening-upward trends. Foraminifera are abundant and include bathyal benthic fauna. This suggests that the channel serves primarily as a conduit for transporting the coarser sediment downslope and that only suspended sediments are delivered overbank to build up the fan's marginal areas.

Sites drilled on the lower fan revealed a surprisingly high abundance of sand deposited by density currents. Individual sand layers range in thickness from a few centimetres up to 10 m and contain a sparse fauna of shallow water origin. Well logs were run at three of the lower fan sites and provide the means for determining accurate sand percentages. The computed total sand percentage of the logged interval is 59.5 per cent. It is apparent that the coring process selectively preserves the finer-grained intervals, as the sand percentage based on the recovered sediments is only 15.6 per cent.

Palaeontologic determinations suggest that the youngest fan lobe was deposited during the late Wisconsin glacial stage with depositional rates ranging from 6.5 to 11.7 m per 1,000 year. These extremely high depositional rates indicate that very large quantities of material have been removed from the shelf and transported to the Mississippi Fan in a short geologic time period. The upper and middle fan channel was sufficiently deep (minimum depth 221 m) to transport all the coarse material within its levees. Part of the coarse-grained load became deposited in the thalweg as part of the aggradational sediment wedge. However, the density flows were thicker than the channel depth such that large quantities of fine-grained material spilled over the confines of the channel to build levee and overbank deposits. At the termination of the channel on the lower fan, the turbidity currents decreased in velocity, spread out of the relatively shallow channels, and deposited their sediment loads in oblong lobes.

In conclusion, the combined results of core data, well logs, and palaeontologic information collected on Leg 96 provide a better understanding of the depositional processes, sediment distributions, and timing of the construction of the Mississippi Fan. These results can now be applied both to other large deep sea fans and, by using our new understanding of the present as a key to the past, to ancient rock equivalents. □

The scientific party aboard *Glomar Challenger* for Leg 96 of the Deep Sea Drilling Project, International Phase of Ocean Drilling, consisted of: Arnold H. Bouma (Gulf Research & Development Co., Houston, Texas; Co-Chief Scientist), James M. Coleman (Louisiana State University, Baton Rouge, Louisiana; Co-Chief Scientist), James Brooks (Texas A&M University, College Station, Texas), William Bryant (Texas A&M University, College Station, Texas), Richard Constans (Chevron USA Inc., New Orleans, Louisiana), Michel Cremer (University de Bordeaux, Talence, France), Laurence I. Droz (Laboratoire de Géodynamique Sous-Marine, Villefranche-Sur-Mer, France), Toshio Ishizuka (University of Tokyo, Tokyo), Mahlon C. Kennicutt (Texas A&M University, College Station, Texas), Barry Kohl (Chevron USA Inc., New Orleans, Louisiana), William R. Normark (US Geological Survey, Menlo Park, California), Suzanne O'Connell (Lamont-Doherty Geological Observatory, Palisades, New York), Mary Parker (Florida State University, Tallahassee, Florida), Kevin Pickering (University of London, London), Claudia Schroeder (Dalhousie University, Halifax, Nova Scotia), Charles E. Stelling (Gulf Research & Development Co., Houston, Texas), Dorrik Stow (Edinburgh University, Edinburgh), William E. Sweet (Mineral Management Service, Metairie, Louisiana), Andreas Wetzel (Geologisches Institut, Tübingen), Jean K. Whelan (Woods Hole Oceanographic Institution, Woods Hole, Massachusetts), Audrey Wright (DSDP, Scripps Institution of Oceanography, La Jolla, California).

Applied molecular biology

Lignin biodegradation becomes biochemistry

from Paul Broda and Alistair Paterson

AFTER some years of increasing interest, rapid progress is now being made in the understanding of lignin biodegradation. Research in a number of laboratories has culminated in the isolation by T.K. Kirk and Ming Tien of a purified enzyme able to depolymerize lignin¹. This enzyme, isolated from the white-rot fungus *Phanerochaete chrysosporium*, is extracellular, oxidative and requires H₂O₂ as a cosubstrate. It is able to cleave a range of lignin models consisting of lignin monomers joined by bonds found in the polymer, and the reactions catalysed by the enzyme are similar to those thought to take place during lignin degradation. The range of bonds cleaved shows that the enzyme has the wide specificity necessary to explain the fungal degradation of lignins. Thus lignin research is evolving from the study of the chemistry of lignin breakdown products and the physiology of white-rot fungi to analysis of enzyme systems, allowing the entry of the molecular geneticists. The genes involved in lignocellulose degradation in fungi and their regulation are of both fundamental and commercial interest.

Lignin has always been a neglected polymer. It is considered difficult to study because, in contrast to cellulose, it lacks steric regularity and monomer chirality. The structure follows from the mechanism of lignin biosynthesis, in which three *p*-hydroxycinnamyl alcohols — coniferyl, sinapyl and coumaryl alcohols — are dehydrogenated to form phenoxy radicals which then spontaneously polymerize. Lignins are very variable, with softwoods and hardwoods having distinctive monomer proportions; grass lignins have in addition phenolic acids bound to the polymer by ester bonds. Such variability of composition means that the incidence of different bonds in lignin varies from one plant to another. The most important is the β aryl ether bond, between the propanoid side chain of one alcohol and the aromatic ring of a second. Many other C—O and C—C bonds have been described including biphenyl linkages between aromatic rings.

The student of lignin biodegradation was in deep water until 1974. In that year two laboratories, those of Kirk (Forest Products Laboratory, Madison, Wisconsin) and Eriksson (Swedish Forest Products Research Laboratory, Stockholm) began to concentrate on *P. chrysosporium* and another white-rot fungus, *Sporotrichum pulverulentum*, which have been recently shown by DNA hybridization methods to be closely related. These laboratories have

encouraged much research, aided by the development by others of techniques for specifically labelling both natural and synthetic lignins with ¹⁴C. Initially much effort was spent on understanding the physiology of lignin degradation in these fungi. Kirk *et al.* established that ligninolytic activity in *P. chrysosporium* was not inducible, but was evoked by the exhaustion of available carbon, nitrogen or sulphur in the growth medium². It is thought that the organism may obtain no net energy from lignin breakdown but only resulting access to new sources of carbohydrate. Kirk and coworkers also showed that lignin degradation was optimal in an atmosphere of 70 per cent oxygen. Eriksson and his research group showed that *Sporotrichum* has, as well as hydrolytic enzymes, a number of oxidative enzymes of cellulose metabolism and a multitude of intracellular catabolic pathways for utilizing lignin breakdown products^{3,4}.

By 1980 it had been established that attack on lignin was oxidative, extracellular, non-specific with regard to substrate, and a secondary metabolic (idiophasic) activity, but no plausible enzymic mechanism had emerged. Hall (Virginia State University, Blacksburg) then suggested that instead an activated oxygen species is the immediate depolymerizing agent⁵. Rapidly, Reddy and coworkers (Michigan State University) and Kutsuki and Gold (Oregon Graduate Centre) provided data suggesting that the hydroxyl radical OH^{*} was produced by *Phanerochaete* specifically during ligninolytic activity^{6,7}. This radical could, in theory, perform the reactions observed to take place during biological attack on lignins but is very difficult to study specifically because of its dynamic relationship with other activated oxygen species. However, it is known that it can be generated by the interaction of H₂O₂ with Fe²⁺ (ref. 8). A number of groups then added H₂O₂ to culture fluids and observed that the reactions obtained with lignin models were similar to those observed to take place in intact cultures⁹. The enzyme that Ming Tien and Kirk have now purified is a haem protein and its activity is inhibited by scavengers of active oxygen species.

A number of questions and possibilities have emerged. Is the depolymerization brought about directly by activated oxygen species such as OH^{*}, or is there a genuine enzyme-substrate interaction? That *Phanerochaete* is also able to decolourize dyes during the ligninolytic phase¹⁰ supports the former hypothesis. The non-

specificity of OH[•] attack is attractive in explaining why there is no need for the stereospecificity and substrate chirality normal for enzyme-substrate interactions. How is the H₂O₂ generated by the fungus? Higuchi and his coworkers (Wood Research Institute, Kyoto University) have shown that alcohol oxidases in other fungi can generate H₂O₂ from lignin models¹¹ whereas Forney *et al.*, have suggested that a H₂O₂ generating system is produced intracellularly by the organism and is active in the fungal periplasm¹². A third possibility is that it is generated in carbohydrate metabolism by glucose oxidase and similar oxidative enzymes¹³.

To the molecular geneticist the field is now open. Eriksson's¹⁴ and Gold's¹⁵ groups have isolated and studied mutants of *Phanerochaete* with altered ligninolytic and cellulolytic activities. In parallel Shoemaker's (Cetus) and Knowles's (Technical Research Centre of Finland, Helsinki) groups have reported on the cloning of hydrolytic cellulases from another filamentous fungus, *Trichoderma reesei*^{16,17}. Analysis of the *Trichoderma* cellobiohydrolase I gene sequence suggests that the codon usage is different from that in yeast. This and the lack of reports of expression

of filamentous fungal genes in heterologous hosts may mean that the structure of genes in these organisms is distinctive. The enzyme described by Ming Tien and Kirk will be a prime target for cloners since lignin degradation is only observed in thin-layer static cultures. If 'ligninases' could be produced in large quantities by submerged culture, lignin research would have made the leap into the biotechnological age. □

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Immunology

Antigens associated with H-2, embryos and tumours

from Elizabeth Simpson

ONE of the most remarkable findings about mouse MHC class I genes cloned from genomic DNA is the extraordinarily large number of them, of which only a minority correspond to the genes of the classic *H-2K*, *D* and *L* loci. Examination of mice of the *H-2^d* strain, BALB/c, revealed 36 class I genes of which 5 mapped to the *K*, *D* and *L* loci and the remainder lay telomeric to *L*, in the *Qa/Tla* region¹. In C57BL/6 (*H-2^b*) mice only 3 of 26 class I MHC genes map to *K* and *D* (the *H-2^b* haplotype does not have an *H-2L* molecule); the remainder are located in the *Qa/Tla* region².

The functions of the classic *K*, *D* and *L* class I *H-2* molecules are well defined: they are guidance molecules for certain T-lymphocyte functions³. *K*, *D* and *L* molecules are extremely polymorphic (there are more than 100 alleles of *K* and *D*). Graft rejection responses and primary mixed lymphocyte reactions are stimulated strongly by allelic differences at these loci but their physiological role appears to lie rather in their being used as restriction elements, through which T lymphocytes (particularly cytotoxic T cells) can recognize and eliminate viruses⁴.

There is less information about the possible roles for the numerous *Qa/Tla* genes and indeed there is evidence for cell surface expression of only some of them⁵.

They are certainly far less polymorphic than *K*, *D* or *L* molecules and allelic differences at certain loci give rise to much weaker graft rejection responses. Thus they have been termed 'medial' transplantation antigens⁶. Those whose alleles can be defined by transplantation reactions can also be identified by cytotoxic T cell responses generated in mixed lymphocyte cultures from T cells taken from mice previously immunized *in vivo*. Very interestingly, these cytotoxic T-cell responses, unlike those to viruses or to minor transplantation antigens (much weaker cell surface antigens, and not encoded by *H-2* linked genes) are not *H-2* restricted by *K*, *D* or *L* *H-2* molecules and this puts them in a class apart⁶. Two medial transplantation antigens whose structural genes are known to lie within the *Qa/Tla* region are *Qed-1*⁷ and *Mta*⁸, and these antigens are expressed on all mature lymphocytes⁶. The relationship of these to the *Qa/Tla* antigens defined serologically is not altogether clear⁶. Since *Tla* antigens were defined serologically and are expressed on thymocytes and lymphoid tumours in certain mouse strains and as *Qa1* and *Qa2* antigens were found serologically to be present on sub-populations of normal lymphoid cells, it was proposed that antigens of the *Qa/Tla* region were

differentiation antigens (see ref. 9).

The hypothesis that *Qa/Tla* region class I antigens might be differentiation antigens predicts that some of them might not be generally expressed in normal adult tissue. This makes the report of Bridell and colleagues¹⁰ in this issue of *Nature* (see p. 756) of very great interest. They have isolated from an SV40 transformed fibroblast line of BALB/c (*H-2^d*) origin, a cDNA clone pAG64 which includes a unique 1.6 kb sequence having remarkable homology with class I MHC genes. Detailed sequence analysis shows that the gene is not that for *K^d*, *D^d* or *L^d*, implying that it is a *Qa/Tla* gene, although mapping of the gene has not been formally established. Expression of this gene is not found in the untransformed parental fibroblast line, nor in normal spleen tissue. Expression is, however, found not only in the SV40 transformed fibroblast line, but in mouse fibroblasts transformed by polyoma virus (another DNA tumour virus), the retroviruses, Rous sarcoma virus and Abelson murine leukaemia virus, and those transformed by the carcinogen methylcholanthrene. In non-fibroblast cells the only normal tissue in which expression of this gene can be detected is thymus, but a variety of tumour cells show evidence of expression, including an AKR thymoma, the mastocytoma P815, a myeloma, a neuroblastoma and a hepatoma. Together this adds up to an impressive body of evidence that the expression of this class I MHC gene has something to do with the process of transformation.

The evidence linking expression of this class I MHC gene with normal differentiation comes from studies with embryonal carcinoma cells which maintain their potential for differentiation under appropriate circumstances. They uniformly fail to express classic *K*, *D* and *L* molecules, but are positive when probed with a subclone of pAG64, revealing the 1.6 kb transcript. This suggests that expression of this transcript may be part of the normal differentiation process, and should be found in embryonic tissue of the appropriate developmental stage. That such an antigen may be capable of eliciting graft rejection comes from studies of rejection of allogenic embryonal carcinoma cells which appear to express *H-2* linked transplantation antigens which are not the serologically detectable *K*, *D* or *L* alloantigens¹¹. However, in genetically identical, syngeneic hosts embryonal carcinoma cells are not rejected¹¹ so the possession of the syngeneic antigen(s) does not render the cells immunogenic, although it may be a marker for the undifferentiated/transformed state.

The immunological argument raised by Brickell *et al.*¹⁰ is that immune responses may be raised to their class I molecule whose expression is repressed after an early stage in embryogenesis, and which therefore does not confront the developing and mature immune system.

Tolerance to it is therefore not induced, that is, it is not regarded as a 'self' molecule. They propose that when re-expression occurs at or after transformation, an antitumour response might well be mounted against the tumour by the host. This could be via natural killer cells, as suggested by the authors, but probably also by T cells, since the response shows memory¹¹⁻¹³ and *Qa/Tla* region encoded molecules can certainly be recognized by T cells⁶⁻⁸. Such *H-2* linked class I antigen(s) are prime candidates for the oncofetal

antigens described by Medawar on the basis of finding that immunization with syngeneic tissue from mid-term embryos¹² or thymus¹³ (interesting in view of the findings of this paper) protects mice from subsequent challenge both with certain transplantable tumours and from the induction of tumours by the injection of methylcholanthrene. □

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Neuropharmacology

Endogenous opioid peptides and pain mechanisms: a complex relationship

from C.J. Woolf and P.D. Wall

ALTHOUGH an ever-increasing number of pharmacologically active peptides are being found in neurones of the central nervous system, it is still not proving possible to find out precisely what neurotransmitter or neuromodulator functions they perform. This may not be so surprising when one remembers how many years it took from Loewi's discovery of acetylcholine to the time when Katz was able to demonstrate unequivocally that it was the transmitter at the vertebrate myoneural junction. The complexity of the central synapse, with the co-existence of more than one transmitter in a single neurone, multiple pre- and post-synaptic receptors, fast and slow postsynaptic potentials, reciprocal inputs, and actions at sites distant from that of release, enormously multiplies the problem of assigning distinct functions to the different neuropeptides.

Of all the peptides, it was the endogenous opioid peptides that excited the greatest attention when they were discovered¹ — and quite rightly because it seemed likely that their action would be related to pain mechanisms. What is the position now, eight years after that discovery?

At least 18 active opioid peptides have been identified which are derived from three large polypeptide precursors². Post-translational proteolytic processing of these inactive precursors liberates a mixture of shorter chain peptides which fall into three families; the β -endorphin-like compounds, the enkephalins and the dynorphins. The different opioids have

varying affinities for the three classes of opiate receptor; the μ , δ and κ receptors. By good fortune, an opiate receptor antagonist, naloxone, exists which has a high affinity only for μ receptors. The dose of naloxone must be increased 10–15 times to affect other opiate receptors.

Although the opioid peptides are widely distributed in the central nervous system, the midline nuclei of the brainstem and the superficial laminae of the dorsal horn of the spinal cord (the substantia gelatinosa) have received particular attention as possible sites for the involvement of the opioids in pain mechanisms. The reason for this selection is that small amounts of morphine or the opioids injected locally into these areas produce analgesia³. The brainstem nuclei are the site of origin of descending inhibitory systems which terminate in the substantia gelatinosa, which is also the site of termination of the small diameter high threshold primary afferent neurones. There is therefore a local opioid system in the spinal cord as well as an input from a brainstem opioid system. Whether opioid action in the spinal cord is pre- or post-synaptic or both is the subject of some controversy⁴. Opiate receptors are present on the terminals of fine afferents in the dorsal horn⁵ but intensive anatomical searches have failed to demonstrate enkephalinergic processes synapsing with these terminals⁶. Because pharmacological and electrophysiological evidence points to a presynaptic action of opioids^{7,8} it is possible that these peptides, like LHRH in the sympathetic ganglion⁹,

operate in a spatial domain that extends beyond the 'classical' synapse. Devastating for the interpretation of the action of opioid agonists or antagonists is the recent demonstration that enkephalin containing boutons contact neurones which themselves contain enkephalin¹⁰. Even if opioids produce inhibition the net effect could be excitation through disinhibition.

In spite of this complexity, it could be argued that because opiate receptor agonists like morphine produce a predictable analgesia, there must be some relation between the endogenous opioids and the expression of pain. The most obvious test of this is to examine pain states in the presence of naloxone. Because of the simplicity of this test, there is a vast literature. The results are however, chaotic. Each positive result is matched by negative ones. We will only review the literature of naloxone effects on man since it is only in humans that pain can be directly reported. In animals, where pain is inferred from a deviation in observable behaviour, the relationship to pain is questionable. Only the most recent papers will be cited. The reader who wishes to go further should work his way back in time through the bibliographies.

The different experiments rarely duplicate each other exactly and there are variations in dose and the use of double blind controls. Of seven papers on experimental pain in normal subjects, four report that naloxone has no effect but three find that naloxone produces hyperalgesia¹¹. Acupuncture analgesia is found to be decreased by naloxone in three studies but not affected by the drug in the most recent and carefully controlled study¹². The analgesia resulting from segmental transcutaneous nerve stimulation is reported to be unaffected in five papers but reduced in one other¹³. Placebo analgesia is reported to be abolished by naloxone in one study, affected only on the third trial, or not at all in two other papers¹⁴. Hypnotic analgesia has not been found to be reduced¹⁵. The analgesia resulting from stimulation of the midbrain is reported to be reduced by two groups and not at all by another¹⁶. Congenital insensitivity to pain was not influenced in three studies but was reversed in one¹⁷.

Truth in science is not judged by majority vote. It is quite clear that the expected simple answer is not apparent. The hodge-podge of conflicting results cannot be explained by the use of inadequate doses since many of the negative studies used higher doses than the positive ones. It is obvious that there must be an uncontrolled naloxone-reversible variable unrelated to pain in these studies, which may well be the degree of the subjects' stress¹⁸.

An alternative approach has been to relate the amount of endogenous opioids in cerebrospinal fluid (CSF), to the patient's pain state. We will not review the literature concerned with opioid levels in plasma,

because these peptides are likely to be released from the pituitary and adrenal medulla with little access to the CNS across the blood brain barrier. Unfortunately, these more direct studies have also produced contradictory results. Acupuncture has been reported separately to increase β -endorphin in CSF²⁰ but is not affected by naloxone. Midbrain stimulation has been found to raise enkephalins in one study and β -endorphin in another, but a third finds that the elevation in β -endorphin occurs even when no analgesia results¹⁶. Chronic pain has been found to be associated with a decreased level of a dynorphin-like function by one group while another has reported no change in β -endorphin or enkephalin levels¹⁹. Congenital insensitivity to pain has been linked to a very high level of β -endorphins with no naloxone effect in one patient and the exact opposite in another¹⁷. In post-operative pain the level of dynorphin-like peptides has been found to be negatively correlated with the amount of pethidine required for pain relief²¹. Finally patients with APUD tumours or Nelson's syndrome with extraordinarily high levels of β -endorphin have normal pain thresholds which are not affected by naloxone²².

We are therefore faced by a fascinating and serious problem. The opioid peptides are clearly present in several populations of neurones and can be released from them in certain situations. On the basis of a vast literature of neuropharmacological experiments in animals it is very likely that the released opioids alter the function of some neurones. The administration of high doses of β -endorphin into the CSF²³ and the intravenous injections of a stable enkephalin analogue²⁴ certainly produce analgesia in man. Why then is the subject in such an obvious mess?

Essentially, the answer seems to be that it was far too naive to expect that a complex family of compounds would have a single function. This attitude developed from the assumption that there are brain areas and systems with labelled functions, for example, 'pain pathways', operating via single excitatory or inhibitory chemicals. An examination of the distribution of the opioids in the brain shows the futility of such wishful thinking: the highest concentration of substance P and the enkephalins are found not in 'sensory' areas but in the basal ganglia²⁵. We know, moreover, that the brain specializes in homeostatic mechanisms which hold systems stable by a number of parallel alternative tactics. It may be that the many negative results originate from the brain's ability to restore a stable state when it is disturbed by a chemical blockade of one of its systems.

The model that has been adopted to study the relation between opioid peptides and pain is still that which was used by Loewi to study acetylcholine; it demonstrates the release of the transmitter in a defined situation and shows that an antagonist blocks the predicted function,

in that situation. This model is not appropriate for studying an exceedingly complex sensory disturbance involving millions of synapses. We cannot use humans as a bioassay in the same way as an isolated strip of vas deferens smooth muscle and expect similar answers. Complex problems do not necessarily have simple solutions. In spite of this though, we can and must, continue to make hypotheses about the possible functional roles of the neuropeptides. There are many crucial aspects of the action of peptidergic systems which we are just beginning to discover and which exemplify their complexity and subtlety. Most of these studies have been performed on sympathetic ganglia and include demonstrations that the peptides work on long-latency, long-action ion channels which do not initiate action potentials but modify the firing behaviour of the postsynaptic neurone²⁶. It may be therefore that the manifest failure to assign a clear role for the endogenous opioids has occurred because we have been looking too crudely, at too short a time course, for a classical neuro-

transmitter-type function, which the compounds simply may not have. □

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Astronomy

The decline of a superstar?

from M.G. Edmunds

ASKED for the mass of the biggest known star, the astronomer-in-the-street would probably reply with an estimate between 60 and 100 $M_{\odot}$, that is, between about sixty and one hundred times the mass of the Sun. Or at least he would have done until a few years ago, when studies of the heart of the giant glowing gas region 30 Doradus in the nearby Large Magellanic Cloud galaxy suggested the presence of a much larger star, perhaps as big as 2,500 $M_{\odot}$. Recent optical studies of the region are somewhat ambiguous, as will be discussed below, but seem to be indicating that it is not necessary to invoke anything larger than normal stellar masses to explain the region's physical properties.

Massive stars (say above 25 $M_{\odot}$) are rare. This is partly because their extremely short lifetimes of a few million years limit their existence to regions of active star formation, and partly because of the small number of massive stars formed, relative to low mass stars, when an interstellar cloud fragments and collapses. The basic physics of the fragmentation process in star formation remains obscure. One can argue that the fragmentation of a cloud is a hierarchical process, and that hierarchical processes (for example, turbulence, or the kind of fragmentation produced on dropping a pane of glass) tend to result in a few large structures and increasingly many small structures until a limit imposed by some physical process occurs. But interstellar cloud fragmentation is a rather special and complicated process, with competing gravitational, turbulent, centrifugal, magnetic,

thermal, radiative and chemical effects occurring. In fact, the actual amount of material which ends up in stars at the end of a burst of star formation is observed to be only a small fraction (perhaps five per cent) of the total mass of the cloud, and elementary models of star formation based on a simple probabilistic fragmentation sequence dominated by a single physical mechanism are almost certainly too simple. Two reasonable physical mechanisms have been proposed, however, to give a limit on the upper mass of a star that can form from a condensing fragment.

The first limiting mechanism is the stability of a star. Radiation pressure becomes an important means of support as stellar mass increases, but when it dominates then the star becomes unstable to radial pulsation. So it has been suggested that any star above 100 $M_{\odot}$ would be unstable and break up. But rotation of the star can help to stabilize it, and it is not anyway clear that pulsations would grow sufficiently large to disrupt the evolution of the star if it had only a short lifetime.

The second mechanism derives from consideration of the collapse of an isolated gas sphere. Studies predict that a core forms, containing about half the mass of the star which will eventually emerge, and that the other half of the mass subsequently accretes onto this core. Since the core will be dense and hot it should produce energy by nuclear reactions as well as by liberation of gravitational potential energy where the infalling gas hits the surface of the core. The resulting strong radiation from the

core will exert radiation pressure on the dust in the accreting material. Calculation¹ suggests that above about $60M_{\odot}$ the pressure transmitted to the gas through collision with the grains should be enough actually to halt the inflow. Thus a star could grow no further.

Given these limiting mechanisms it is not surprising that considerable excitement greeted the report of observation of a $2,500M_{\odot}$ in the 30 Doradus nebula by Cassinelli *et al.*² in 1981. These authors strengthened the previous suggestion of Feitzinger *et al.*³ that the central part of the nebula contained an object of $250\text{--}1,000M_{\odot}$. The basic arguments in favour of such a large star were fourfold; the implied ultraviolet flux required to ionize the nebula, the characteristics of the ultraviolet absorption spectrum (as observed by the IUE satellite), the surface brightness on the sky where the object is situated (known as R136 from a Radcliffe Observatory catalogue), and the suspected intrinsic variability. The ultraviolet flux required to power the nebula after other hot stars had been taken into account suggested a need for the equivalent of 30 very hot (O3) stars within the, apparently stellar, R136 image. The ultraviolet spectrum suggested a single star with high velocity (up to $3,400\text{ km s}^{-1}$) mass outflow. The surface brightness of the R136 image suggested a luminosity far in excess of that expected from a $100M_{\odot}$ star, and the variability implied a single object rather than many. Further support for a single star was given by speckle interferometry⁴, that ingenious technique of using the instantaneous structure of images (which are inevitably blurred by terrestrial atmospheric motions) to set upper limits on the size of an unresolved object. The suspected size limit (set by the aperture of the telescope) was less than, or possibly just resolved at 0.02 arc seconds. The expected diameter of a $2,500M_{\odot}$ star would be much smaller than this (about 2×10^{-5} arc seconds), but even at 0.02 arc seconds a cluster of normal stars providing the nebular ionization would have to be surprisingly dense.

Along with a spirited and updated restatement⁶ of the arguments for the presence of a very massive star, two papers have appeared^{4,5} claiming that the region is really not so peculiar. The ultraviolet spectrum, it appears, is not too unusual and could be produced by the superposition of the spectra of a few hot O-type stars and a bright Wolf-Rayet star. Wolf-Rayet stars (of which several are found elsewhere in the nebula) are very hot stars which show a particular broad-line emission spectrum with chemical peculiarities. They are reasonably well understood as massive (but certainly less than $100M_{\odot}$) stars which have been stripped down towards their core by mass loss. They have lost hydrogen, in particular, and show the products of nuclear processing, possibly often involving the simultaneous evolution of (and mass exchange with) a binary companion. Exotic

objects indeed, but still within the accepted stellar mass range.

The interpretation of the surface brightness of R136 depends critically on the assumed absorption by dust in the nebula, and between the nebula and Earth. Its surface brightness (as limited to finite size by the Earth's atmosphere) may in fact be less than that which a cluster of stars NGC 3603 in our own Galaxy would have if it were at the same distance as R136. The cluster NGC 3603 is clearly resolved into stars simply because it is nearer to us. A reassessment of photometry, and new photometric observations, suggest that R136 is not variable — removing the necessity of a single coherent object. Even the speckle interferometry result is cast into doubt. An independent study⁷ has resolved the object into at least two stars — and indeed this double was actually resolved visually (at about 0.5 arc second separation) by an indefatigable observer of double stars, Innes⁸, working in South Africa in the 1920s. The resolution of such small scale structure, below the typical single image size of 1–2 seconds of arc caused by the smearing by the atmosphere, is possible either on nights of exceptional atmospheric conditions or by the eye and brain acting as a sort of real-time auto-correlator of the image structure. The eye can respond to what used to be called 'the image within the image'; effectively a doublet structure within the transient speckles making up the image structure. The failure of the other speckle interferometry group to resolve the source as double may have been due to the particular design of their image processing system.

Further investigation of the image structure will be essential, since the physical extent of R136 is crucial to the arguments for or against a single star. If the majority of the luminosity really comes from a source smaller than 0.02 seconds of arc then the single-object hypothesis would be hard to resist, both because of the abnormally high density implied for the required star cluster and the resulting very short implied dynamical lifetime⁶ (of order only 1,000 years!) for such a dense cluster. If the region really has its luminosity spread out in several objects over an image of say 0.5 to 2 seconds of arc, then the cluster hypothesis is preferable. Unfortunately, the interpretation of speckle interferometry of almost anything other than simple point or double sources is a difficult (and at present unsolved) problem for astronomical objects which are relatively faint when viewed from Earth.

One other important question does not seem to be entirely settled, and that is the number of normal stars needed to supply the ultraviolet radiation required by the observed nebular re-emission. The Wisconsin group⁶ maintain that some 30 stars are required, but Melnick⁴ suggests that stars outside R136 can supply all but 30 per cent of the ionizing flux and that only five to eight hot O stars are actually re-

quired inside R136. This brings us back to Walborn's original suggestion⁹ that R136 is just a dense star cluster of hot, but not exceptional, stars.

The recent results are perhaps something of a disappointment. Very massive stars (which eventually become black holes) built up by agglomeration in a dense star cluster are popular models for active galactic nuclei. A single black-hole-plus-accretion-disc model for R136 is probably not viable both because it is an extended, rather than a strong point-like, X-ray source and (more importantly) because of its star-like optical and ultraviolet spectrum. It could be argued that the radiation-pressure-on-dust mechanism for stopping very massive star formation might not work so well in the Magellanic Clouds where heavy element abundances, and certainly the abundance of dust formed from such elements, is lower than in our own Galaxy. A favourite device of theorists considering star formation during the formation and early evolution of galaxies has been to suggest that much more massive stars were able to form when overall abundance of the heavy elements was low. Studies of other galactic systems with low abundances and the chemical evidence in old stars in our own Galaxy may eventually help to establish whether very massive stars (other than in galactic nuclei) are more than a theoretician's dream, but the *prima facie* case for observation of such a star in the Magellanic Clouds seems to be weakening under cross-examination. □

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100 years ago

In a letter dated Tokio, October 3, Prof. James Main Dixon writes: — "During the two or three days at the end of August we enjoyed fine dry weather, but the sun was copper-coloured and had no brightness. When we got to Nikko, the people came to us to inquire if some catastrophe were impending, for the appearance of the sun foreboded evil. We laughed at their fears, and assured them all was right. However it seems that if the appearance of the sun foreboded no evil, it was a wonderful sign of the greatest earthquake and volcanic catastrophe on record. The fearful explosion of Krakatoa, took place on August 26, and there seems little reason to doubt that the monsoon had carried the volcanic dust along with it, the dust obscuring the sun. The distance is nearly 3000 miles."

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Cancer and cell senescence

SIR — In their *News and Views* article "Step-by-step into carcinogenesis"¹, Cairns and Logan discuss the finding that oncogenic DNA can transform immortal established cell lines, such as mouse 3T3, but not diploid cells which have limited growth potential. They write that: "One of the built-in programmes protecting the whole organism against invasion by clones of uncontrolled variants is the programmed senescence that normally comes into play after a certain number of cell generations. This barrier has to be overcome whenever a cancer is formed or a cell line is established *in vitro*, and so it is not illogical to expect that transfection with any established cell line could show what changes are needed to circumvent programmed senescence". These are astonishing assertions based, as far as I am aware, on no direct evidence.

The hypothesis that the senescence of diploid cells is a protective device to prevent the indefinite spread of malignant cells, which was first proposed by Dykhuizen², runs into several serious difficulties. Human diploid fibroblasts have a growth potential of about 60 population doublings. Starting with a single cell, this represents 2⁶⁰ cells, that is 10¹⁸ cells equivalent to approximately 10⁶ kilograms of cells. It is clear that a clone of cancer cells with this, or any similar, proliferative capacity could easily kill the organism, whether or not it finally underwent senescence. This is clearly illustrated in the case of avian cells. It is well established that normal chicken cells can be transformed with oncogenic viruses to produce highly tumorigenic derivatives. Very few, if any, of these malignant strains have been passaged indefinitely, either *in vivo* or *in vitro*, yet they can kill the animal, before the "barrier of senescence" is reached. In addition, skin fibroblasts from certain inherited cancer-prone syndromes (such as Bloom's syndrome, ataxia telangiectasia, Fanconi's anaemia and Werner's syndrome) have a significantly shorter growth potential than cells from normal individuals³.

If senescence is programmed, why should it be accelerated in these conditions, and if it is a barrier, why are the affected individuals not protected from metastatic growth of tumours?

Apart from the possibility suggested by Cairns and Logan that normal cells have programmed senescence and tumour cells do not, there are quite different explanations for their differences in growth potential. For example, finite growth versus infinite growth may be attributed simply to heterogeneity in the growth potential of individual clones.

As pointed out by Orgel⁴, for a population with indefinite growth it is only necessary for each dividing cell to produce

on average more than one viable daughter. It is therefore quite possible for a permanent line to contain many sub-clones which have limited proliferative potential, whilst retaining a "stem line" of potentially immortal cells. In populations of diploid cells, such a stem line may be lost during serial sub-culture⁵. Another possibility is that transformed cells have reverted to a de-differentiated, quasi-embryonic or germ-line state, and that they are able to avoid, or protect themselves from, the accumulation of defects in macromolecules, which may be responsible for the ultimate demise of differentiated somatic cells^{6,7}.

The demonstration of the relationship between transformation and infinite growth potential in rodent cells is of obvious importance, not least because it reinforces the view that a full understanding of the processes leading to the emergence of tumour cells may also depend on unravelling the mechanisms of cellular senescence.

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CAIRNS AND LOGAN REPLY — In the first half of his letter, Dr Holliday gives three reasons for thinking that programmed cell senescence is not a protection against the development of cancer. We believe that each of the arguments is fallacious.

The common human cancers, as described in textbooks of pathology, are full of terminally differentiated cells. Indeed, they often have a smaller proportion of cycling cells than the normal tissues from which they have arisen (perhaps for the trivial reason that a cancer lacks the structural organization that allows a tissue such as an epithelium to discard its differentiated progeny). So we have no notion how many divisions the stem-line has to undergo in order to produce a fairly small cancer containing, say, 2³⁰ cells, save that it will be a lot more than 30 divisions. For all we know, clones that start uncontrolled growth may often find themselves permanently arrested because their stem-line hits 60 divisions. Certainly, many more invasive clones arise — and can be found in serial sections — than give rise to endlessly proliferating cancers.

The tumorigenic derivatives of normal chicken cells transformed by certain onco-

genic viruses produce tumours that grow by recruiting host cells rather than by clonal expansion (the way most human cancers grow). Programmed senescence will never be a barrier to this kind of growth.

As for the inherited cancer-prone conditions such as Bloom's syndrome, we know that they raise the rate of production of certain kinds of genetic variants, and so we might expect them to be associated with an increased incidence of cancer. In the absence of accelerated senescence they might have shown an even higher rate.

Two of the three papers that we discussed in our *News and Views* article describe how cancerous clones, produced by transfection of normal rodent fibroblasts with certain oncogenes, become invasive and embark on unrestrained growth; but in the end the growth of the clones invariably ceases unless the cells have also undergone immortalization. These papers, published in the same issue of *Nature*, provide direct evidence that programmed senescence is a protection against cancer.

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"Microgravity"

SIR — The European Space Agency is currently inviting life scientists to participate in their Microgravity Research Programme to study effects attributable to "the abolition of gravitational influences" in an orbiting spacecraft. It should be pointed out, however, that it is not the gravitational field that is "abolished" in orbit. Indeed, if it were, there would be no orbit, no curvature of the flight-path. The relevant feature of the free-fall conditions of orbital flight is that objects can retain their relative position within the spacecraft without developing stresses (*g*-forces) in their mechanical supports.

When a weight is supported by a spring, the spring tension is a stress phenomenon, not a gravitational one. In orbit, it is the stress distribution, not the gravitational field, that is different from that familiar on the Earth's surface. The "*g*-forces" are stress phenomena in spite of the fact that the unit of acceleration, *g*, used to characterize them is defined in a gravitational context. For orbital conditions it would be preferable to use the expression "micro-*g*" in place of "microgravity". This might avoid the temptation to think in terms of field effects and might direct attention more appropriately towards the rearrangements of molecular architecture associated with stress gradients.

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Dating the Crucifixion

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The date of the Crucifixion has been debated for many years, but there has been no agreement on the year nor the day. Astronomical calculations have now been used to reconstruct the Jewish calendar in the first century AD and to date a lunar eclipse that biblical and other references suggest followed the Crucifixion. The evidence points to Friday 3 April AD 33 as the date when Jesus Christ died.

THE only certainty about the date of the Crucifixion is that it occurred during the 10 years that Pontius Pilate was procurator of Judaea (AD 26–36). Nearly every year in this period has its advocates (see, for example, ref. 1) while the day of the execution is also uncertain since there appears to be a difference of one day between the date given by the Gospel of John and that indicated by the Synoptics: Matthew, Mark and Luke.

In what follows we have set out to reconstruct the Jewish calendar in the first century AD, improving on the accuracy of previous versions. We have sought to reconcile the documentary evidence that exists with our reconstruction of the Jewish calendar and we have used calculations of the occurrence of a lunar eclipse which, if accepted, allow the day, month and year of the Crucifixion to be determined precisely.

Biblical evidence

Tacitus² agrees with the four gospels that the Crucifixion took place during the period when Pontius Pilate was procurator of Judaea, between AD 26 and AD 36. All four gospels agree that Jesus died a few hours before the beginning of the Jewish sabbath (nightfall on a Friday) and—within a day—that it was the time of the Passover, the annual Jewish feast held at the time of a full Moon.

This evidence alone compels us to reject many of the dates suggested in the past. Thus, one of the earliest traditions, going back to Tertullian (AD 200), gives the date as 25 March AD 29. This date was not accepted everywhere throughout the early church and we now know from astronomical calculations that the time of the Passover Moon in AD 29 was in April, not March.

Passover time was precisely specified in the official festival calendar of Judaea, as used by the priests of the temple (see, for example, ref. 3). Lambs were slaughtered between 3 p.m. and 5 p.m. on the 14th day of the Jewish month Nisan (corresponding to March/April in our calendar). The Passover meal began at moonrise that evening, at the start of 15 Nisan (the Jewish day running from evening to evening) (*Leviticus* 23, 5; *Numbers* 28, 16). John's Gospel differs from the other three in stating that the day of Jesus' trial

and execution was that before Passover (*John* 18, 28 and 19, 31), on 14 Nisan. The precise interpretation of the Synoptics is less unambiguous. Here, briefly, are three of the many possible interpretations which have been proposed.

(1) A straightforward reading of the Synoptics indicates that the Last Supper was a Passover meal, eaten at Passover time (in the evening at the start of 15 Nisan), with the Crucifixion occurring later that Jewish day, on 15 Nisan (see for example *Mark* 14, 12). This disagrees with John's date of 14 Nisan (see *Jeremias*⁴).

(2) Many scholars, however, have suggested that the Last Supper described by the Synoptics was not strictly a Passover meal but that Jesus, knowing of his imminent arrest, held a Passover-like meal on the evening before (see *Luke* 22, 15). Certainly the Synoptics do not mention the eating of a Passover lamb, and this interpretation is in broad agreement with the Johannine account in which the farewell meal is explicitly stated to have occurred before the feast of Passover (*John* 13, 1). The timing also agrees, so that on this reading all four gospels give 14 Nisan as the Crucifixion date. (Variations on this basic interpretation are discussed in refs 3, 5 and 6.)

(3) Jaubert⁷ has proposed that the Last Supper reported by the Synoptics was a strict Passover meal but held at Passover time as calculated using the 'sectarian' calendar followed by the Qumran community and others (see ref. 8). On this reading, the Last Supper was held on Tuesday evening, at the start of the Jewish Wednesday (the sectarian calendar Passover day, and recorded by the Synoptics), the Crucifixion was on Friday (all four gospels) and the official

Passover was on Saturday (recorded by John), in which case all four gospels again give 14 Nisan (official calendar) as the date of the Crucifixion.

Thus some scholars believe that all four gospels place the Crucifixion on Friday, 14 Nisan and others believe that, according to the Synoptics, it occurred on Friday, 15 Nisan. For generality, we assume at this stage that both dates are possible and set out to determine in which of the years AD 26–36 the 14th and 15th Nisan fell on a Friday. Previous attempts (see for example refs 4, 9–12) to use astronomy to resolve this ambiguity have shown that while the times of new and full

IMAGE
UNAVAILABLE
FOR
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REASONS

A full-page miniature from a thirteenth-century English manuscript from Salisbury, now at All Souls College, Oxford. (Courtesy Bodleian Library.)

* Also Jesus College, Oxford (G.J.H.) and Department of Astrophysics, Oxford (W.G.W.).

Moons can be accurately specified, we do not know with what skill the Jews of the first century could detect the first faintly glowing lunar crescent after conjunction with the Sun. (The new Moon itself is invisible by definition.)

Jewish calendar

Hitherto it has been customary to assume arbitrarily that the sickle of the new Moon would be invisible to the unaided eye until a certain length of time (usually 30 hours) had elapsed since conjunction. Fotheringham's more realistic criterion, based on the apparent position of the Moon in the sky at sunset, was modified and improved by Maunder¹², but even that criterion is not rigorous, excluding several thin crescents that have been observed.

We have therefore computed the visibility of the lunar crescent as a function of time after sunset for the beginning of each lunar month in the period of interest. Whether or not the crescent Moon is visible depends on whether its contrast with the sky background exceeds the visual contrast threshold¹³. The lunar semidiameter and the position of the Moon in the sky at and after sunset have been evaluated from harmonic syntheses of the perturbed orbits of the Earth and Moon and the sky brightness for an observer at Jerusalem calculated as a function of the depression of the Sun below the horizon, as is the Moon's apparent surface brightness. At the latitude of Jerusalem, we find that the lunar crescent is first visible after sunset at a lunar altitude corresponding to approximately 0.5° less than that given by Maunder and this is consistent with many recent observations of the first sickle of the new Moon. Assuming normal atmospheric transparency, we obtain the results of Table 1.

Although in the first century AD the beginning of the Jewish lunar month (in the official calendar) was fixed rigorously by

astronomical observation, difficulties arise because of the Jewish use of intercalary (or leap) months. Twelve lunar months total approximately 11 days less than a solar year, but for agricultural and ritual purposes, lunar months were kept at roughly the same place in the solar year by the intercalation of a thirteenth month when necessary, roughly once every three years. In the first century AD, intercalation was regulated annually by proclamation by the Sanhedrin according to certain criteria^{4,8,9,14}, one of the most important of which was that Passover should fall after the vernal equinox. If, towards the end of a Jewish year, it was estimated that Passover would fall before the equinox, the intercalation of an extra month before Nisan was decreed. Table 1 has been constructed on this basis.

Unfortunately, a leap month could also be decreed if the crops had been delayed by unusually bad weather (since the first fruits must be ripe for presentation on 16 Nisan) or if the lambs were too young. There are, however, no historical reports of the proclamation of leap-months in the years AD 26–36, so that it is possible that in some years Nisan was one month later than given in Table 1. Calculations show that in the period AD 26–36, if Nisan was one month later than given in Table 1, 14 Nisan would not fall on a Friday in any year and 15 Nisan would fall on a Friday only in AD 34 (April 23).

Table 2 lists all the possible dates of a Friday Crucifixion falling on either 14 or 15 Nisan. These are the only dates that are astronomically and calendrically possible for the Crucifixion. We now consider which of them can be eliminated by means of other available evidence.

Further evidence

AD 27 is almost certainly too early. *Luke 3*, 1–2 carefully states that John the Baptist began his ministry in the fifteenth year of Tiberius Caesar and subsequently baptised Jesus. Depending on whether the Hellenistic (Roman) civil or the Jewish ecclesiastical reckoning is used, the fifteenth year (=340 Seleucid Era) would either have been autumn AD 28–29 or spring AD 29–30 (see ref. 15). In addition, most scholars believe that Pilate had been procurator for some time before the Crucifixion (see *Luke 13*, 1 and 23, 12).

Similarly, AD 34 is almost certainly too late, for it would conflict with the probable date of Paul's conversion. We can fairly confidently date the later events in Paul's life and, working back from these using time intervals given by Paul himself (for example, see *Galatians 1*, 18 and 2, 1) leads many scholars to infer Paul's conversion was in AD 34 (for example, see ref. 4). Moreover, AD 34 is only a possible Crucifixion date if the weather that spring had been exceptionally severe. There is therefore no positive evidence in favour of AD 34 and we exclude it. (The only eminent advocate of 23 April, AD 34 that we have come across is Sir Isaac Newton, whose chief reason seems to have been that 23 April is St George's Day.)

Having eliminated AD 27 and AD 34, we note from Table 2 that the Crucifixion must have occurred on 14 Nisan and that the interpretation of the Last Supper as a Passover meal held at the official time cannot be correct.

We remark that by this means, a scientific argument has been used to distinguish between different theological interpretations of the nature of the Last Supper. We have also shown that the Crucifixion occurred on 14 not 15 Nisan, so that Jesus died at the same time as the Passover lambs were slain. This is consistent with many New Testament statements such as "Christ our Passover is sacrificed for us" (1 *Corinthians 5*, 7).

By elimination, AD 30 and AD 33 are now the only two plausible dates for the Crucifixion. The earliest possible time at which Jesus can have begun his ministry is autumn AD 28 (see ref. 15) while John's gospel records three different Passovers occurring in the ministry (including that at the Crucifixion). If this evidence is accepted, AD 30 cannot be the Crucifixion year and AD 33 is the only possibility.

This is also consistent with the reference in *John 2*, 20 which records that the Jews said to Jesus at the first Passover of his ministry that the temple had taken 46 years to build. Assuming

Table 1 The date of 14 Nisan in Jerusalem, AD 26–36

Year (AD)	New Moon time		Deduced date of 14 Nisan	
26	6 April	6:40	Sunday	21 April
27	26 March	20:05	Thursday	10 April*
28	15 March	2:30	Tuesday	30 March
29	2 April	19:40	Monday	18 April†
30	22 March	19:55	Friday	7 April†
31	12 March	0:25	Tuesday	27 March
32	29 March	22:10	Sunday	13 April*
33	19 March	12:45	Friday	3 April
34	9 March	5:25	Wednesday	24 March
35	28 March	6:10	Tuesday	12 April
36	16 March	17:50	Saturday	31 March

The time of new Moon is given as calculated apparent (sundial) time of conjunction for Jerusalem (± 5 min). The deduced date is the Julian day (from midnight to midnight), starting at 6th hour 14 Nisan and ending at 6th hour 15 Nisan.

* 14 Nisan AD 27 and AD 32 could have been on the following day if the new Moon was not detected due to poor atmospheric transparency.

† In each of these cases it is not impossible, but highly improbable, that 14 Nisan would have occurred on the preceding day.

Table 2 Calendrically possible dates for the Crucifixion

Jewish day	Source	Date (Julian calendar)
14 Nisan	John's Gospel and Synoptics (2, 3)*	Friday 11 April AD 27†
		Friday 7 April AD 30
		Friday 3 April AD 33
15 Nisan	Synoptics (1)*	Friday 11 April AD 27†
		Friday 23 April AD 34‡

* Synoptics (1, 2, 3) refers to the three possible interpretations in the text.

† There is some uncertainty, depending on the atmospheric conditions, as to whether this day was on 14 or 15 Nisan (see text and Table 1). We include all possibilities for completeness.

‡ Only in the case of a leap month being inserted because of exceptionally severe weather (see text).

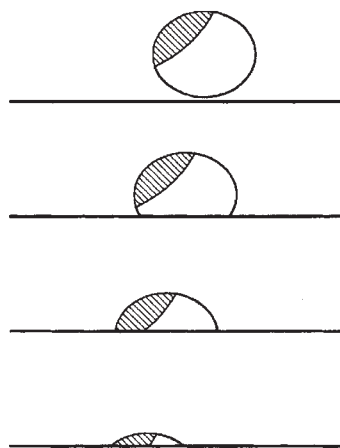


Fig. 1 Moonrise on Friday 3 April AD 33 as seen from Jerusalem. The effects of atmospheric refraction have been included and give rise to the distorted shape of the Moon. The time interval between successive diagrams is 45 s. The most probable colours of the Moon were: shaded area, red; unshaded area, yellow-orange.

that this refers to the inner temple (see ref. 1), the 46 years leads to AD 30 or 31, depending on how much preparation time was involved before building began. If the only Passovers of Jesus's ministry were the three explicitly mentioned in John's gospel, an AD 33 crucifixion implies a ministry of about 2½ years. Many scholars, however, believe that John omitted to mention a further Passover, in which case the ministry would have lasted for 3½ years.

On the two dates possible on calendrical grounds, scholarly opinion appears divided with some authors^{5,16} preferring 7 April, AD 30, and others^{3,14} 3 April, AD 33. We now consider further evidence that has not, to the best of our knowledge, been used in helping to date the Crucifixion—the subsequent occurrence of a lunar eclipse.

We first take up the meaning and significance of the references to the Moon being “turned to blood” in the Bible and also in the Apocrypha.

In *Acts* 2, 14–21 it is recorded that on the day of Pentecost, the apostles were accused by a crowd of being drunk and that Peter stood up and said “No, this is what was spoken by the prophet Joel: In the last days, God says, I will pour out my spirit on all people... I will show wonders in the heavens above... The Sun will be turned to darkness and the Moon to blood before that great and glorious day of the Lord shall come.”

Table 3 Lunar eclipses visible from Jerusalem AD 26–36

Date*	Day†	Magnitude‡	Time eclipse started
15 Aug. AD 26	Friday	50%	23.16
31 Dec. AD 27	Wednesday	70%	23.45
14 June AD 29	Tuesday	Total	20.45
9 Dec. AD 29	Friday	45%	20.92
25 April AD 31	Wednesday	35%	21.58
19 Oct. AD 31	Friday	25%	4.82
3 April AD 33	Friday	60%	Occurred at rising Moon
27 Sept. AD 33	Sunday	85%	4.88
11 Feb. AD 35	Friday	55%	4.91
7 Aug. AD 35	Sunday	60%	20.30
31 Jan. AD 36	Tuesday	Total	Occurred at rising Moon
26 July AD 36	Thursday	Total	22.23

* Julian Calendar.

† Julian day (from midnight to midnight as distinct from the Jewish day).

‡ Fraction of the area of the Moon covered at the midpoint of the eclipse.

It is not clear whether Peter was claiming that all the quoted prophecy from Joel had recently been fulfilled (for example ref. 17) or whether the words refer to the future, but in our view, the phrase “the Moon turned to blood” probably refers to a lunar eclipse, in which case the Crucifixion can be dated unambiguously.

Peter prefaces his quotation from Joel with the words “Let me explain this to you... this is what was spoken by the prophet Joel”. He appears to be arguing that recent events had fulfilled the prophecy he was about to quote. If this interpretation is correct “the last days” began with Christ's first advent (see also 1 Peter 1, 20; *Hebrews* 1, 1–2) and the outpouring of the spirit (v. 17–18) commenced at Pentecost and “that great and glorious day” (v. 20) refers to the Resurrection. “The Sun will be turned to darkness” (v. 20) refers back to the 3 hours of darkness which occurred only 7 weeks previously, at the Crucifixion (*Matthew* 27, 45), and would be understood as such by Peter's audience.

As is well known, the mechanism by which the Sun was darkened may have been a khamsin dust storm. Since the darkened Sun occurred at the Crucifixion it is reasonable to suppose that “the Moon turned to blood” occurred that same evening, “before that great and glorious day”, the Resurrection. This interpretation of *Acts* 2, 20 is supported by F. F. Bruce¹⁸.

Other documentary evidence suggests that on the day of the Crucifixion, the Moon appeared like blood. The so-called ‘Report of Pilate’, a New Testament Apocryphal fragment (see ref. 19), states that at the Crucifixion “the Sun was darkened; the stars appeared and in all the world people lighted lamps from the sixth hour till evening; the Moon appeared like blood”. Although much of the Apocrypha cannot be used as primary historical evidence, Tertullian records that Pilate wrote a report of all the events surrounding the Crucifixion for the Emperor Tiberius. The manuscript fragments of the ‘Report of Pilate’ are all of later date, but may be partly based on the lost original¹⁹. On the other hand, the report may have used the *Acts* as a source, in which case it may be significant that the event described by Peter in which the Moon turned to blood is clearly stated to have occurred at the Crucifixion. It is of course also possible that the report is a late Christian ‘forgery’, but even such a document may be thought to reflect a widely held contemporary belief. In other words, the Report of Pilate is secondary supporting evidence that the Moon appeared like blood on the evening of the Crucifixion.

The Moon turned to blood

The reason an eclipsed Moon is blood red is well known. Even though the Moon is geometrically in the Earth's shadow, sunlight still reaches it by refraction in the Earth's atmosphere and is reddened by having traversed a long path through the atmosphere where scattering preferentially removes the blue end of the spectrum. Descriptions of some well documented ancient eclipses have been compiled by Ginzel²⁰ and matched with calculated eclipse dates. We quote three examples: (1) The lunar eclipse of 20 September 331 BC occurred 2 days after Alexander crossed the Tigris and the Moon was described by Curtius (IV, 10 (39), 1) as “suffused with the colour of blood”. (2) The lunar eclipse of 31 August AD 304 (probably) which occurred at the martyrdom of Bishop Felix, was described in *Acta Sanctorum* “when he was about to be martyred the Moon was turned to blood”. (3) The lunar eclipse of 2 March AD 462 was described in the Hydatius Lemicus Chronicon thus: “on March 2 with the crowing of cocks after the setting of the Sun the Full Moon was turned to blood”.

In the mediaeval European annals compiled by Pertz²¹, many lunar eclipses are described by “the Moon turned to blood”, while Stephenson²² considers that the prophecy of Joel (2, 31) clearly alludes to a lunar eclipse. It is therefore surprising that the link between the Crucifixion and a lunar eclipse does not appear to have been made before, although Bruce²³ approaches this conclusion.

Lunar eclipses visible in Jerusalem AD 26–36

We have determined the eclipses relevant to our work by the use of the most comprehensive data available²⁴ as corrected by Stephenson^{25,26} in the light of Babylonian records and long-term changes in the Earth's rate of rotation. All lunar eclipses (total and partial) visible from Jerusalem between AD 26 and AD 36 are listed in Table 3 which shows that in the period AD 26–36, there was only one lunar eclipse at Passover time visible from Jerusalem. The date, Friday, 3 April AD 33, is the most probable date for the Crucifixion deduced independently using other data. The interpretation of Peter's words in terms of a lunar eclipse is therefore not only astronomically and calendrically possible, but it also allows us with reasonable certainty to specify Friday, 3 April AD 33 as being the date of the Crucifixion. The random probability of a lunar eclipse occurring at moonrise on a particular date is, of course, small. We remark that in 1899 Chambers²⁷ noted that 3 April AD 33 coincided with a full Moon and "that full Moon suffered eclipse, but she emerged from the Earth's shadow about a quarter of an hour before she rose from Jerusalem" (D. Hughes, personal communication). Presumably this eclipse was considered irrelevant to the date of the Crucifixion since it was believed to be invisible from Jerusalem. However; the more accurate calculations presented here prove that this eclipse was visible.

Visual appearance

Calculations show that this eclipse was visible from Jerusalem at moonrise. (All times quoted below are local Jerusalem times as measured by a sundial, and the probable error in the eclipse times is about ± 5 min.) The start of the eclipse at 3.40 p.m. was invisible from Jerusalem, being below the horizon. At its maximum at about 5.15 p.m., with 60% of the Moon eclipsed, the eclipse was still below the horizon from Jerusalem. The Moon rose above the Jerusalem horizon at about 6.20 p.m. (the start of the Jewish Sabbath and also the start of Passover day in AD 33) with about 20% of its disk eclipsed and the eclipse finished some 30 min later at 6.50 p.m.

Although at moonrise only 20% of the total area (πr^2) of the Moon's disk was eclipsed (in the umbral shadow), this "bite" was positioned close to the top (leading edge) of the Moon. Figure 1 shows the appearance of the Moon at, and shortly after, moonrise on 3 April AD 33. As the umbral shadow (in which the Sun is geometrically entirely hidden) was near the top of the Moon, about 65% of the visible area of the rising Moon would initially have been seen as fully eclipsed (Fig. 1) while the remainder would have been in the penumbral shadow.

The coloration of eclipses varies greatly with atmospheric conditions. For partial eclipses, particularly with the Moon at high altitude, there is a large contrast difference between the obscured and unobscured part of the disk so that the Moon often appears almost white with a very dark "bite" removed. However for some partial eclipses the red colour of the umbral shadow is clearly visible. For example, Davis²⁸ has recently depicted in colour an eclipse sequence as seen by the human eye with the Moon low in the sky, when the blood red of the umbra in the partial eclipse phase is almost as vivid as when the eclipse is total.

For the eclipse of 3 April AD 33, the Moon was just above

the horizon and the most probable colour of the visible portion would have been red in the umbral shadow (shaded in Fig. 1) and yellow-orange elsewhere. The small yellow-orange region would have indicated that the Moon had risen, but most of its visible area would have "turned to blood". If in fact a massive dust storm was responsible for darkening the Sun a few hours previously, dust still suspended in the atmosphere would have tended to modify these colours, probably further darkening and reddening the Moon.

The eclipse of 3 April AD 33 would probably have been seen by most of the population of Israel, since the Jews on Passover Day would be looking for both sunset and moonrise in order to commence their Passover meal. Instead of seeing the expected full Paschal Moon rising they would have initially seen a Moon with a red "bite" removed (Fig. 1). The effect would have been dramatic. The Moon would grow to full in the next half-hour. The crowd on the day of Pentecost would undoubtedly have understood Peter's words as referring to an eclipse which they had recently seen.

Conclusions

In ancient times eclipses (total or partial) were regarded as supernatural. Thus, the first century Jewish historian Flavius Josephus records that a lunar eclipse occurred on the same night as Herod the Great burnt alive Matthias and some other Jews for sedition. (This was a partial eclipse according to Ginzel²⁰.) The lunar eclipse on the same night as the Crucifixion would similarly have been interpreted by many as a supernatural sign, and may well have been an important factor influencing the overnight change of mind of the Jews and Pilate towards the body of Jesus, leading to the placing of a military guard on the tomb.

The lunar eclipse may also be relevant to the well-known reference to a Crucifixion solar eclipse in some translations of *Luke 23*, 44–45 ("It was now about the sixth hour, and darkness came over the whole land until the ninth hour: the Sun was eclipsed"). Solar eclipses are of course impossible at Full Moon and in any case last for minutes, not hours. Only five early (but major) manuscripts of *Luke* refer to this solar eclipse, but it may be that a scribe copying an original *Luke* text, and knowing of an oral tradition of an eclipse at the Crucifixion, wrongly amended the text to refer to a solar eclipse.

At first sight, it might be thought curious that a Crucifixion lunar eclipse is not mentioned in the gospels. Although at the time of the Crucifixion this eclipse would have seemed of great significance, and indeed Peter apparently referred to it about 7 weeks later, and Pilate may have referred to it in his original report to Tiberius, in retrospect this lunar eclipse would have seemed insignificant to the gospel writers compared with the Crucifixion and the Resurrection. The gospel writers were not primarily interested in providing clues for chronologists.

We thank Mr J. G. Griffith for information on some New Testament manuscripts and for detailed discussions, and Dr F. R. Stephenson for lunar eclipse data and some important astronomical references. We also thank the following for their comments on a first draft of this paper: Dr O. R. Barclay, Dr G. A. D. Briggs, Mr O. Edwards, Canon J. Fenton, Dr P. E. Hodgson, Professor H. F. D. Sparks, Dr G. Vermes, Dr D. Wenham and Dr D. E. H. Whiteley.

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Composition measurements of tropospheric ions

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Tropospheric ions are of considerable interest as they have the potential to induce aerosol formation and because in situ measurements of their composition are a powerful tool for neutral trace gas detection. The first measurements of tropospheric ion compositions are now reported.

ATMOSPHERIC ions control the electrical properties of the atmospheric medium but also have other physical, chemical, and possibly even biological effects. Among these, aerosol formation through ion nucleation and the potential influence of ions on trace gases through ion-molecule or ion-catalysed reactions may be important¹⁻⁴.

Atmospheric ions are also important for analytical and diagnostic applications. It has been found^{5,6} that *in situ* ion composition measurements can be used as a powerful tool for neutral trace gas detection. This method has already provided new information on stratospheric trace gases such as CH₃CN, H₂SO₄, HSO₃ and HNO₃. Of these, H₂SO₄ and HSO₃ appear to be of special importance as they are the major gaseous precursors of stratospheric aerosols which are thought to influence the climate^{4,7}.

Besides providing information on trace gases, atmospheric ions may also lead to new insights into aerosol and nucleation processes. Ion clusters seem to develop properties which are midway between those of molecules and condensed matter⁷. In fact, larger ion clusters resemble very small solution droplets.

In view of their significance it is important to identify the chemical nature of atmospheric ions. So far, however, this has been possible only for the altitude region above ~15 km using rocket-⁸ and balloon-borne^{6,9-18} mass spectrometers. As yet no experimental method other than *in situ* mass spectrometry could be used to provide detailed information on the chemical nature of atmospheric ions. Ion mobility spectroscopy is not sufficiently selective for the large ion clusters existing in the denser atmospheric regions, the stratosphere and troposphere. Mass spectrometry, however, becomes increasingly difficult as the ambient atmospheric gas pressure rises.

We have developed and built an aircraft-borne mass spectrometer for tropospheric and lower stratospheric ion composition measurements. We report here on the negative ion data obtained in the two flights of this instrument with an emphasis on analytical applications.

Instrumental technique and measurements

The experimental method used is similar to that used by our group for balloon-borne stratospheric ion composition measurements^{6,7,9,10}, so that only the instrumental modifications specific to aircraft-borne measurements will be discussed here.

The cryogenically pumped quadrupole mass spectrometer (QMS) is attached sideways to a cylindrical probing vessel (PV) which, in turn, is mounted inside the mid-section of the aircraft fuselage. An air intake mounted ~10 cm above the fuselage carries the sample through a stainless steel tube (diameter, 4 cm; length, 133 cm) to the PV. Having passed the PV the sample leaves the fuselage through an exit tube of the same size.

Inside the PV, ions contained in the air stream are electrically drawn to the QMS-sampling electrode (diameter, 6 cm) the central part of which carries an inlet hole (diameter, 0.01 cm) and to which a bias potential between -100 and +100 V can be applied.

Two flights were performed with the twin jet research aircraft *Falcon* of the DFVLR (Deutsche Forschungs- und Versuchsanstalt für Luft- und Raumfahrt). The flights took place on 28 April 1983 (flight F1) and 17 May 1983 (flight F2) during daytime over the FRG.

Negative ion mass spectra using narrow band modes (mass resolving modes) of the QMS were obtained at 11.7 km (F1) and between 8.2 and 11.2 km (F2) with local tropopause levels being at 11.4 and 11.3 km, respectively.

A detailed description of the instrumental technique, the circumstances of the measurements and positive ion data obtained in the same flights will be published elsewhere¹⁹.

Negative ion composition

A typical negative ion mass spectrum obtained at 10.75 km altitude (F2) is shown in Fig. 1. It contains one large peak at 188 AMU and several smaller peaks (125, 223, 80, 160, 251, 286, 297 AMU). The CEM-dark count rate was ~0.2 s⁻¹ and thus a 2σ confidence limit excludes higher mass 'peaks' from being significant.

Using a 2σ significance criterion for mass peak search, one finds from all mass spectra the masses compiled in Table 1. Mass uncertainties quoted are mostly due to the relatively low mass resolution setting used. Total negative ion count rates measured are ~15 s⁻¹ which represents a fraction of ~5 × 10⁻⁷ of the total maximum ion flux to the QMS sampling electrode. This relation is consistent with laboratory simulation studies. Tentative ion identifications are given in Table 1. Most of the observed ions can be grouped into two families, NO₃⁻(HNO₃)_n and HSO₄⁻(HNO₃)_n, which is similar to the lower stratosphere ion composition¹⁴.

Hydrates of the above ions also seem to be present. Figure 2 shows the total fractional count rates for NO₃⁻ and HSO₄⁻ clusters. At the higher altitudes NO₃⁻ clusters are dominant whereas HSO₄⁻ clusters become comparably abundant at the lower altitudes. Figure 3 shows fractional count rates for individual ion species. The ion NO₃⁻(HNO₃)₂ is dominant at the upper heights while its hydrate becomes most prominent at the lowermost altitude.

Discussion

The equilibrium size distribution of NO₃⁻(HNO₃)_n clusters can be calculated using laboratory thermodynamic data²⁰, a typical nitric acid vapour abundance²⁰ of 2 × 10⁸ cm⁻³ and a measured atmospheric temperature of 218 K (11 km). Resulting fractional abundances for the members of the NO₃⁻(HNO₃)_n family (total NO₃⁻(HNO₃)_n equals 100% are: <1 × 10⁻¹⁰% (n = 0), 0.3%

Table 1 Masses and maximum fractional count rates for negative ions observed in flights F1 and F2 at altitudes between 8.2 and 11.7 km

Mass	Maximum	Ion
62±3	22.2	NO ₃ ⁻
80±3	3.8	NO ₃ H ₂ O
125±2	22.2	NO ₃ HNO ₃
143±3	2.4	NO ₃ HNO ₃ ·H ₂ O, (NO ₃ HSO ₃) ⁻
160±3	15.0	HSO ₄ HNO ₃
188±2	66.7	NO ₃ (HNO ₃) ₂
209±3	22.6	HSO ₄ (HNO ₃) ₂ H ₂ O; HSO ₄ ⁻ HSO ₃ H ₂ O; HSO ₄ ⁻ HSO ₃
223±2	11.3	HSO ₄ (HNO ₃) ₂
241±3	14.3	HSO ₄ (HNO ₃) ₂ H ₂ O; HSO ₄ HSO ₃ ·HNO ₃
251±3	5.7	NO ₃ (HNO ₃) ₃
273±3	7.5	HSO ₄ (HNO ₃) ₃ H ₂ O; HSO ₄ HSO ₃ HNO ₃ H ₂ O; HSO ₄ HSO ₃ ·HNO ₃
286±3	2.6	HSO ₄ (HNO ₃) ₃
297±3	2.7	HSO ₄ (H ₂ SO ₄) ₂
352±3	2.1	HSO ₄ (H ₂ SO ₄) ₂ HNO ₃

Tentative ion identifications are also given. Note that maximum fractional count rates refer not to a single altitude.

($n=1$), 93% ($n=2$), and 6.7% ($n=3$). For $n=0$ only an upper limit can be estimated because only a lower limit to the bond energy²¹ of NO₃HNO₃ ($-\Delta H^\circ \geq 26$ kcal mol⁻¹) is available. In comparison, the values measured at 11.7 km are: 6% ($n=0$), 12% ($n=1$), 83% ($n=2$) and 6% ($n=3$). Good agreement between observations and model predictions is found for $n=3$ and $n=2$, whereas the values measured for $n=1$ and $n=0$ are far too large.

For $n=0$, the discrepancy can hardly be explained by collisional cluster ion dissociation of $n=1$ during ion sampling for at least two reasons: first, the large bond energy of NO₃HNO₃ does not allow much dissociation. In particular, one should expect the dissociation of NO₃HNO₃ to be very much less efficient than that of NO₃(HNO₃)₂. By contrast, however, the observed ratios NO₃HNO₃/NO₃(HNO₃)₂ and NO₃/

NO₃HNO₃ are not much different, which strongly argues against NO₃ being a fragment of NO₃HNO₃. The second fact supporting this view is that most NO₃ is hydrated (mass 80). Because H₂O ligands are much less strongly bonded²² than HNO₃ ligands, the fragment of NO₃HNO₃H₂O would be NO₃HNO₃ and not NO₃H₂O. Fragmentation of NO₃HNO₃ can, of course, not be followed by hydration of the fragment NO₃ as the fragmentation occurs inside the instrument. In summary, NO₃ and its hydrate cannot be fragments formed by electric field-induced collisional dissociation during ion sampling.

The large NO₃ and NO₃H₂O abundances clearly reflect a non-equilibrium situation. Thermal dissociation of the very stable NO₃HNO₃ must be too slow to compete with further HNO₃ clustering of NO₃HNO₃. Thus, NO₃HNO₃ formation is followed by rapid HNO₃ clustering²³ leading to NO₃(HNO₃)₂. Further HNO₃ clustering is strongly reduced due to efficient thermal dissociation of NO₃(HNO₃)₃. Here a thermodynamic equilibrium between HNO₃ clustering and thermal HNO₃ dissociation is reached. Subsequently, as originally proposed^{7,10}, NO₃(HNO₃)_n clusters are converted to HSO₄(HNO₃)_n clusters by reactions involving H₂SO₄, HSO₃, and possibly also HSO₅.

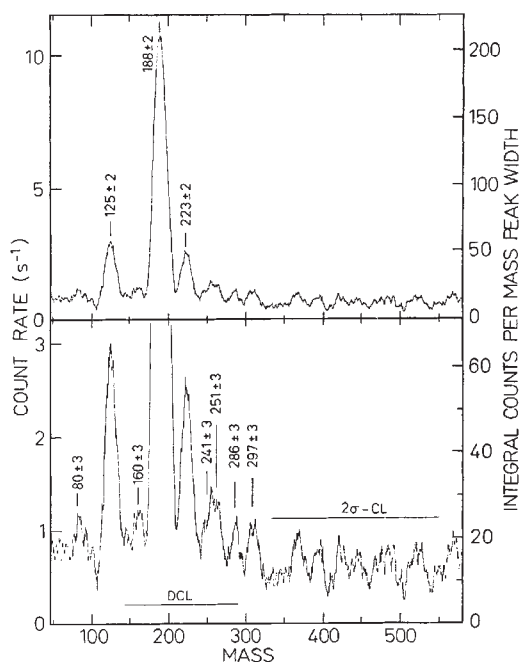
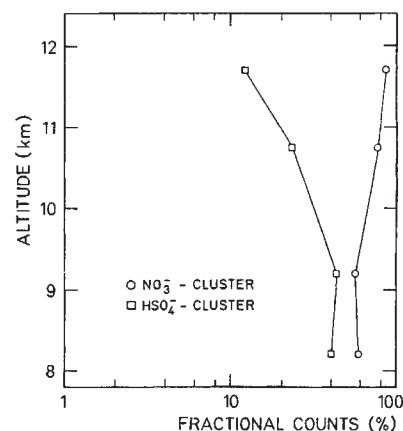
For such a steady-state situation one obtains the following continuity equation:

$$[\text{NO}_3(\text{H}_2\text{O})_n]k[\text{HNO}_3] = ([\text{NO}_3(\text{HNO}_3)_n] + [\text{HSO}_4(\text{HNO}_3)_n])t_R^{-1} \quad (1)$$

where k is the phenomenological two-body coefficient for HNO₃ clustering to NO₃ or the rate coefficient for HNO₃ switching with NO₃H₂O and $t_R = 1/(\alpha n_+)$ is the ion-ion recombination lifetime. The quantities α and n_+ are the ion-ion recombination coefficient and the total positive ion concentration. Taking $[\text{HNO}_3] = 2 \times 10^8$ cm⁻³, $k = 10^{-9}$ cm³ s⁻¹ and a typical^{24,25} $t_R = 100$ s around 11.7 km altitude, one estimates an ion abundance ratio $([\text{NO}_3(\text{HNO}_3)_n] + [\text{HSO}_4(\text{HNO}_3)_n])/([\text{NO}_3] + [\text{NO}_3 \cdot \text{H}_2\text{O}])$ of ~20. Compared with the observed ratio of ~16 the above estimate is in reasonably good agreement, which strongly supports the steady-state concept proposed.

Interestingly, the observed analogous ratio for NO₃HNO₃ and its products of ~10 is not very much different from the above observed ratio for NO₃ and NO₃H₂O of ~20. Therefore it seems that most of the observed large NO₃HNO₃ abundance may also be due to a non-equilibrium situation arising from inefficient thermal dissociation of NO₃(HNO₃)₂. In addition, however, electric field-induced collisional dissociation of NO₃(HNO₃)₂ during ion sampling may contribute, although this effect may be quite small and less than in the stratosphere. For HSO₄(HNO₃)_n clusters laboratory thermodynamic data are not available and hence model predictions cannot be made. From stratospheric ion composition measurements^{13,14}, however, HNO₃ clustering to HSO₄ was found to be weaker than to NO₃.

The present observation of 223/160 being smaller than

**Fig. 1** Negative ion mass spectrum measured in flight F2 at altitudes between 10.5 and 11.2 km. Mass numbers for major peaks are indicated. The dark count level (DCL) and the 2σ confidence level (2σ-CL) are also indicated.**Fig. 2** Total fractional count rates for NO₃⁻ and HSO₄⁻ cluster ions as measured in flights F1 and F2. Only the 11.7-km data relate to F1.

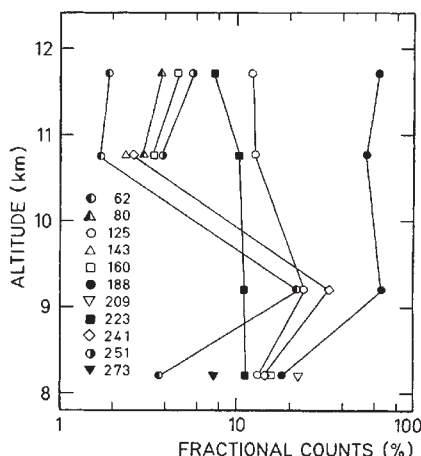


Fig. 3 Fractional count rates for major negative ion species as measured in flights F1 and F2. Only the 11.7-km data relate to F1.

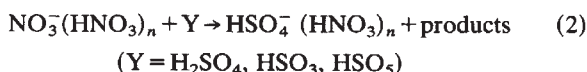
188/125 is consistent with the above finding which supports the proposed identifications of the ions 160 and 223.

Negative ion hydration: Of the observed ions thermodynamic data have been published²² only for $\text{NO}_3^-(\text{H}_2\text{O})_n$. Taking these, a typical water vapour abundance, and a measured temperature of 218 K at 11.7 km, one estimates the following size distribution for the $\text{NO}_3^-(\text{H}_2\text{O})_n$ family: $2.4 \times 10^{-6}\%$ ($n=0$), $1.8 \times 10^{-2}\%$ ($n=1$), 4.8% ($n=2$) and 95.2% ($n=3$). Evidently NO_3^- should be multiply hydrated, which is not observed. This discrepancy might be due to electric field-induced collisional dissociation during ion sampling. In any case, water vapour is sufficiently abundant to allow for very fast hydration within time scales being very much shorter than those for ion-ion recombination or reactions involving HNO_3 .

We have measured hydration data from laboratory studies for $\text{NO}_3^-(\text{HNO}_3)_n$ clusters with $n > 0$, such studies being carried out²⁶ using high-pressure mass spectrometry techniques. The clusters $\text{NO}_3^-(\text{HNO}_3)_n$ revealed a rather unexpected behaviour in that hydration of $\text{NO}_3^-(\text{HNO}_3)_n$ clusters with $n > 0$ becomes more efficient when n increases. This is similar to what has been found recently for $\text{HSO}_4^-(\text{H}_2\text{SO}_4)_n$ clusters from both stratospheric observations⁷ and laboratory studies²⁷. With these recent findings it is likely that the ions 143 ($\text{NO}_3^-(\text{HNO}_3)_2\text{H}_2\text{O}$), 209 ($\text{NO}_3^-(\text{HNO}_3)_2\text{H}_2\text{O}$), 273 ($\text{NO}_3^-(\text{HNO}_3)_3\text{H}_2\text{O}$) and possibly 241 ($\text{HSO}_4^-(\text{HNO}_3)_2\text{H}_2\text{O}$) are hydrates. It might also be possible that some of these ions contain HSO_3 or HSO_5 (see Table 1), however, these identifications are less likely at the upper heights.

If the $\text{NO}_3^-(\text{HNO}_3)_n$ and $\text{HSO}_4^-(\text{HNO}_3)_n$ clusters are strongly hydrated, electric field-induced collisional dissociation of NHO_3 ligands becomes less probable, because the more weakly bonded H_2O -ligands should be preferably 'boiled' off from the cluster internally excited by energetic collisions. Thus, the HNO_3 -shell of the cluster may, indeed, be protected against dissociation. This may explain the close agreement between observed and theoretically predicted abundances for $\text{NO}_3^-(\text{HNO}_3)_2$ and $\text{NO}_3^-(\text{HNO}_3)_3$. A similar argument may apply to lower stratosphere measurements where strong hydration should also be expected.

Fractional abundances of NO_3^- and HSO_4^- clusters: Core ions of the type HSO_4^- are formed from NO_3^- cores by



as originally proposed^{7,10} and as confirmed for H_2SO_4 ($n=1, 2$) by laboratory studies²⁸. For $n=2$ and $\text{Y} = \text{H}_2\text{SO}_4$, a rate coefficient k_2 of $1.1 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ was found and a similar value should apply also to other Y and n under consideration.

Thus, a steady-state treatment yields

$$[\text{NO}_3^-(\text{HNO}_3)_n] k_2 [\text{Y}] = [\text{HSO}_4^-(\text{HNO}_3)_n] t_{\text{R}}^{-1} \quad (3)$$

Possible hydration is not indicated.

Taking $\text{Y} = \text{H}_2\text{SO}_4$ and sulphuric acid vapour abundances as $\sim 10^6 \text{ cm}^{-3}$ around 11.7 km, as given by the model of Hamill *et*

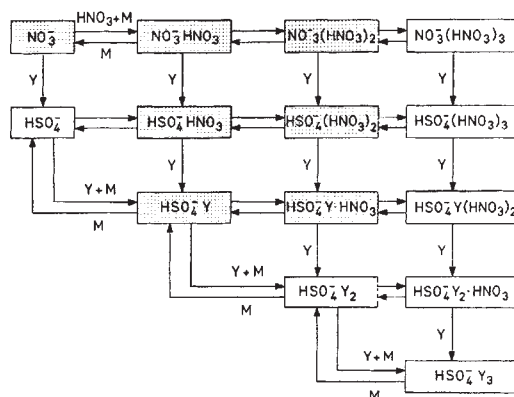


Fig. 4 Simplified reaction scheme for major tropospheric negative ions. Y denotes an acidic sulphur-bearing trace gas ($\text{H}_2\text{SO}_4, \text{HSO}_3, \text{HSO}_5$). Hydration is not included. Acid clusters may be substantially hydrated. M denotes a third body. Horizontal arrows represent HNO_3 clustering and thermal HNO_3 dissociation reactions, respectively. Reaction partners for these processes are indicated only for the first steps.

*al.*¹, an abundance ratio of ~ 0.1 is estimated for HSO_4^- and NO_3^- clusters. This ratio is similar to that observed and supports the proposed identification of HSO_4^- clusters. As t_{R} does not change much with decreasing heights between 11.7 and 8.2 km, the observed increase of the abundance ratio for HSO_4^- and NO_3^- clusters must reflect an increase of $[\text{Y}]$ with decreasing height.

Assuming that Y molecules displace an HNO_3 ligand in $\text{HSO}_4^-(\text{HNO}_3)_n$ clusters on every collision, which implies a rate coefficient of $1 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ for $\text{HSO}_4^- \cdot (\text{HNO}_3)_n$ formation, one obtains

$$\frac{[\text{HSO}_4^- \text{Y}(\text{HNO}_3)_m]}{[\text{HSO}_4^-(\text{HNO}_3)_n]} = \frac{[\text{HSO}_4^-(\text{HNO}_3)_m]}{[\text{NO}_3^-(\text{HNO}_3)_n]} = k_2 [\text{Y}] t_{\text{R}} \quad (4)$$

This represents an upper limit to the left-hand side ratio as the requisite rate coefficient may be even smaller than k_2 and, even more important, because a back-switching reaction may occur.

Thus, $\text{HSO}_4^- \text{Y}(\text{HNO}_3)_m$ ions should have fractional abundances around 11–12 km of only $\sim 1\%$ which is hardly above the detection limit for our measurement. At lower heights, however, the abundance ratio (4) may approach ~ 1 . Here cluster ions containing Y ligands should be detectable.

In fact it is at the lower heights where ions potentially containing Y molecules such as 209, 241 and 273 become prominent. At 8.2 km, for example, the abundance ratio of ions, possibly being identifiable as $\text{HSO}_4^- \text{Y}(\text{HNO}_3)_m$ (209, 241 and 273) on the one and $\text{HSO}_4^-(\text{HNO}_3)_n$ on the other hand, is ~ 1.6 . When compared with the ratio for HSO_4^- clusters and NO_3^- clusters being ~ 0.7 , the above value is similar. Therefore at the lower heights such ions may contain Y ligands.

The following picture of the chemical evolution of upper tropospheric negative ions then emerges: NO_3^- cluster ions are quickly formed²³ from precursor ions arising from galactic cosmic-ray ionization of the atmosphere. Direct precursors of NO_3^- cores are probably $\text{CO}_3^-(\text{H}_2\text{O})_n$ clusters, which are converted²³ to $\text{NO}_3^-(\text{H}_2\text{O})_n$ by reactions involving oxides of nitrogen, in particular, nitric acid. Further reactions (Fig. 4) with HNO_3 lead to $\text{NO}_3^-(\text{HNO}_3)_n$ clusters, which may be markedly hydrated. Thermodynamic equilibria with respect to HNO_3 clustering are not fully established on the low size side of the $\text{NO}_3^-(\text{HNO}_3)_n$ distribution. Ions with $n=0$ and $n=1$ are much more abundant than expected for an equilibrium situation.

The $\text{NO}_3^-(\text{HNO}_3)_n$ clusters are subsequently converted¹⁰ to $\text{HSO}_4^-(\text{HNO}_3)_n$ by reactions involving acidic sulphur-bearing trace gases Y, for example H_2SO_4 and possibly HSO_3 and HSO_5 . Such gases may also cluster to HSO_4^- cluster ions leading to $\text{HSO}_4^-(\text{Y})_l(\text{HNO}_3)_m$ mixed clusters⁷ which again may be markedly hydrated.

Related trace gas detection

Nitric acid: The concentration of nitric acid vapour [HNO_3] is related to the measured ion composition through continuity equation (1). Hence the ion composition measurements can be used as a powerful tool for HNO_3 vapour detection^{29,30}. Basically, two possible methods exist, the so-called steady-state and the equilibrium method. Here we only use the former—it builds on expression (1) and its estimated error is approximately a factor of 2. Resulting nitric acid vapour abundances inferred from the present F1 and F2 data are shown in Fig. 5 along with previous data obtained using chemical filter and IR methods. Theoretical model calculations are also shown for comparison.

Our data bridge the gap between ~7- and 12.5-km altitude where almost no measurements were made previously, except for one IR data point of Evans *et al.*³¹ and an inferred³² upper limit of 10^{-10} . Our volume mixing ratios range between $\sim 6 \times 10^{-12}$ and 6×10^{-10} with an indication of a minimum around 9.2 km altitude. When compared with previous measurements they seem to be distinctly lower than the IR data point. The data points at around 11–12 km agree reasonably well with the model calculations of Fishman and Crutzen (with heterogeneous HNO_3 -removal)³³ and with those of Miller *et al.*³⁴. At lower heights our data are distinctly lower than the model calculations of Fishman and Crutzen.

Our data support the view that upper tropospheric HNO_3 is influenced by a stratospheric source³¹. Around 8–10 km altitude a minimum appears to exist which separates the regions influenced by the stratospheric and tropospheric HNO_3 sources.

The enormous influence of heterogeneous HNO_3 removal is demonstrated by the different model cases A and B of Fishman and Crutzen. As pointed out recently by Turco *et al.*³⁵, dramatic short-term fluctuations of tropospheric HNO_3 vapour may be induced by rainout and washout. In a model simulation these authors show that HNO_3 may be depleted locally by a factor of 10 after strong rainfall. They also pointed out that heterogeneous removal of HNO_3 may significantly affect the composition and acidity of cloud and rain water.

Acidic sulphur-bearing gases: The total concentration of Y gases, such as H_2SO_4 , HSO_3 and HSO_5 , is related to the

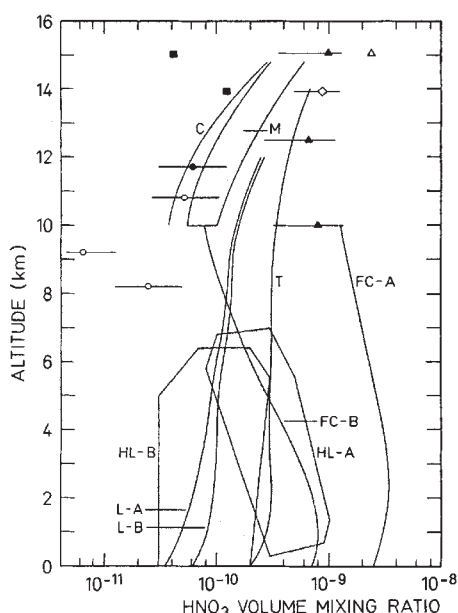


Fig. 5 Inferred nitric acid abundances (●, F1; ○, F2). For comparison previous measurements using chemical filter (■, ref. 37; HL, ref. 38; A, continental northern mid-latitudes; B, remote regions; and IR (▲, ref. 31; ◇, ref. 39; △, ref. 40) methods are also shown. Comparison with theoretical model calculations (C, ref. 21; M, ref. 34; FC, ref. 33; A, without heterogeneous HNO_3 removal; B, with heterogeneous HNO_3 removal; L, ref. 41; T, ref. 35).

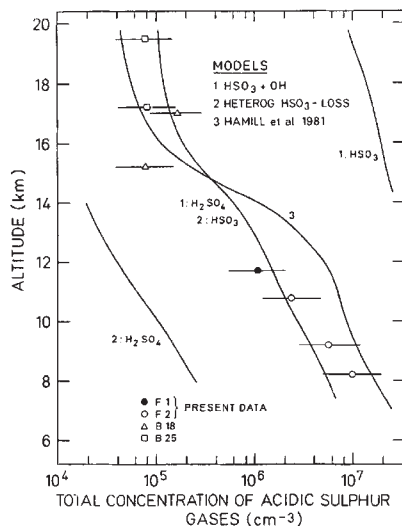
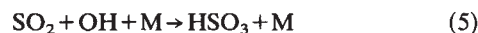


Fig. 6 Inferred total concentrations [Y] of acidic sulphur-bearing gases such as HSO_3 , HSO_5 , H_2SO_4 (F1 and F2 present data; B18 and B25 previous balloon data; ref. 15, and S. Qui and F.A., unpublished data). For comparison previous model calculations¹ for [H_2SO_4] and present model calculations are also shown.

measured abundance ratio for HSO_4^- and NO_3^- clusters (equation (3)). Thus [Y] can be inferred from the measurements using a steady-state approach¹⁵, which again involves an estimated error of a factor of ~2. Resulting [Y] values are shown in Fig. 6 together with previous measurements performed in the lower stratosphere, and theoretical model calculations. The present data increase with decreasing height from $\sim 10^6 \text{ cm}^{-3}$ at 11.7 km to $\sim 10^7 \text{ cm}^{-3}$ at 8.2 km and are distinctly larger than the lower stratospheric values, which scatter around 10^5 cm^{-3} . The abundance of acidic sulphur-bearing gases therefore seems to increase distinctly below the tropopause. Comparison with theoretical models of the tropospheric sulphur cycle is difficult as these are in poor shape therefore the present data may contribute only by elucidating the tropospheric sulphur cycle.

It is generally accepted^{1,35,36} that SO_2 is the most important source of acidic sulphur in the troposphere and that the first step in SO_2 -oxidation is



$$(k_5[\text{M}] = 2 \times 10^{-12} \text{ cm}^3 \text{ s}^{-1}; \text{high-pressure limit})$$

where M is a collision partner, usually N_2 and O_2 . The fate of HSO_3 is uncertain but it is thought to be converted to sulphuric acid, leading to $\text{H}_2\text{SO}_4/\text{H}_2\text{O}$ aerosols. A previous model of Turco *et al.*³⁵ assumed the conversion to proceed by OH reactions leading to SO_3 which in turn is converted to H_2SO_4 through a reaction with H_2O . The [H_2SO_4] profile predicted by this model is described by curve 3 in Fig. 6 which fits the observed [Y] reasonably well. However, the model implies that [HSO_3] is much larger than [H_2SO_4] (curve 1 in Fig. 6) and thus the predicted [Y] would become much larger than the observed one. Obviously, the observations require a much more efficient HSO_3 -sink than the OH-reaction. Potential HSO_3 sinks were recently reviewed by McKee *et al.*³⁶ including reactions with O_2 and H_2O . Unfortunately, however, requisite kinetic data for neither of these processes are available and therefore model predictions cannot be made. If, for example, reaction with O_2 was responsible for the HSO_3 conversion, our inferred upper limit to the HSO_3 lifetime of 10^4 s would set a lower limit to the rate coefficient of $\sim 10^{-22} \text{ cm}^3 \text{ s}^{-1}$. An alternative potential HSO_3 sink⁷ may be heterogeneous HSO_3 removal by aerosols. Then [Y] should be represented by

$$[\text{Y}] = t_A[\text{SO}_2]k_5[\text{M}][\text{OH}] \quad (6)$$

if, as for H_2SO_4 , a sticking probability of one is assumed also for HSO_3 . Then [Y] would be described by curve 2 in Fig. 6 which is consistent with the observations.

Conclusions

The negative ion composition measurements in the upper troposphere revealed the presence of two major ion families, $\text{NO}_3^-(\text{HNO}_3)_n$ and $\text{HSO}_4^-(\text{HNO}_3)_n$. From the measured ion composition new information can be obtained on the trace gases HNO_3 and acidic sulphur-bearing species Y, for example, H_2SO_4 , HSO_3 and HSO_5 .

Inferred nitric acid vapour abundances fill an observational gap between about 7 and 10 km. They support the view that a stratospheric source of upper tropospheric HNO_3 exists.

Inferred Y-abundances have interesting implications for tropospheric nucleation and SO_2 oxidation. As far as nucleation is concerned they suggest that H_2SO_4 may be strongly supersaturated in the troposphere and that therefore thermodynamic requirements for nucleation are easily met. If Y were mostly H_2SO_4 , the saturation ratio at 8 km, for example, could be as large as 1,000.

Kinetic limitations to nucleation are also largely reduced in the middle troposphere due to relatively large Y abundances. Future improved *in situ* measurements of the negative ion

composition may allow us to differentiate between the various HSO_3 loss mechanisms. Measurements such as those reported here have the potential to elucidate the tropospheric and stratospheric sulphur cycles. An interesting point is that the various HSO_3 conversion mechanisms imply different extents of OH-radical consumption³⁶. Consumption of HO_x by SO_2 oxidation would have important consequences for tropospheric sulphate formation and acid precipitation. If operative, it would give rise to a nonlinear response between SO_2 emission rates and sulphate abundances³⁶.

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Isolate-specific S-antigen of *Plasmodium falciparum* contains a repeated sequence of eleven amino acids

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Antibodies raised against a Plasmodium falciparum protein expressed in Escherichia coli reacted with a 220,000-molecular weight antigen of mature blood-stage parasites. The protein resembles the sporozoite surface antigen being composed of tandem repeats of 11 amino acids. However, it is isolate-specific and the encoding gene is not detectable in strains that do not express the protein.

PLASMODIUM FALCIPARUM causes the most severe form of human malaria. Immunity develops slowly after repeated infection over many years, probably due to the large number of antigenically distinct strains and also possibly to sequential expression of different antigens by a single parasite population similar to the antigen variation in the protozoan *Trypanosoma brucei brucei*. Antigenic variation of this type occurs in monkeys infected with *Plasmodium knowlesi*^{1,2}, and with *P. falciparum* infection in the squirrel monkey³.

Antigenic heterogeneity among isolates of *P. falciparum* has been directly demonstrated by two-dimensional gel analysis of

immunoprecipitates and by immunofluorescence using monoclonal antibodies^{4,5}. The S-antigen system first described by Wilson et al.⁶, is extremely diverse. These soluble, heat-stable antigens are present in the sera of some infected individuals and are released into the medium during culture of *P. falciparum* *in vitro*⁷. Although there are many distinct S-antigen types in a restricted geographical area, the S-antigen of a particular isolate is apparently stable long term *in vitro* and *in vivo*^{8,9}. These antigens have been shown unequivocally to be large polypeptides encoded by *P. falciparum*¹⁰ that vary in different isolates and, in many cases, do not cross-react immunologically.

Nothing is known of the molecular basis of their antigenic or size differences, or even of their function(s).

We have reported previously the detection of antigens from the asexual blood stage of *P. falciparum* expressed in *Escherichia coli*¹¹. For this, a cDNA library was constructed in the bacteriophage vector λ Amp 3 derived from the vector λ gt11¹². A Papua New Guinea isolate of *P. falciparum* designated FCQ27/PNG (FC27) was grown in asynchronous *in vitro* culture and mRNA was prepared from a mixture of all stages of the erythrocytic cycle including free merozoites. cDNA prepared from the parasite mRNA was inserted into the *Eco*RI site located 17 codons from the C-terminus of the β -galactosidase gene in the vector. The resulting polypeptides contained 1,004 amino acid residues of β -galactosidase fused to *P. falciparum* polypeptides of variable length. Such fused polypeptides were produced in abundance by an *E. coli* strain that is protease-deficient. Many clones that express *P. falciparum* antigens have been identified by screening with sera from adult individuals living in an area endemic for malaria. We report here the isolation and some properties of one clone that encodes a portion of the S-antigen of FC27.

Antigen from Ag16

cDNA clones constructed in λ Amp3 were screened *in situ* with affinity-purified anti-*P. falciparum* antibodies prepared from sera collected from non-parasitaemic adults who have had life-long exposure to malaria. Many clones were reproducibly positive for antigen expression, and one clone, designated Ag16, is described here. The fused polypeptide produced by induced cultures of Ag16 (designated FPAG16) was characterized by immunoblot analysis. Protein samples from Ag16 were electrophoresed on a polyacrylamide gel, electroblotted onto nitrocellulose and reacted with rabbit anti- β -galactosidase antiserum followed by ¹²⁵I-Protein A from *Staphylococcus aureus*.

Ag16 produced a fused polypeptide of approximately 146,000 molecular weight (MW) that reacted with anti- β -galactosidase serum and also with human antibodies to *P. falciparum*. The fused polypeptide presumably consisted of 116,000-MW β -galactosidase and approximately 30,000-MW *P. falciparum*-encoded sequence (Fig. 1). Sequence studies (see below) confirmed that Ag16 contains a single cDNA insert of 763 base pairs (bp) which could encode 254 amino acids (MW ~ 30,000). FPAG16 is expressed at high levels and can be readily visualized as a dominant band on polyacrylamide gels by Coomassie blue staining (Fig. 1). The fused polypeptide was insoluble in non-denaturing aqueous buffers and was therefore enriched in a 100,000g pellet prepared after the lysis of the cells with lysozyme (Fig. 1c). The fused polypeptide was fractionated by gel filtration in SDS, and obtained in ~80% purity with an estimated yield of 2.5 mg l⁻¹ of log phase, induced culture.

Anti-FPAG16 antibodies in high titre were produced in mice immunized with unfractionated lysates of Ag16 cultures and rabbits immunized with FPAG16 purified as described in Fig. 1. To examine the specificity of the rabbit anti-FPAG16 serum, it was reacted with an array of 78 independently isolated antigen-positive clones including Ag16 on a nitrocellulose filter, using the colony assay described¹¹. The Ag16 colonies reacted very strongly with anti-FPAG16 (Fig. 2a) while all other colonies reacted minimally. As many other colonies are producing fused polypeptides with a β -galactosidase component identical to FPAG16, the *P. falciparum*-encoded portion of the fused polypeptide appeared to be remarkably immunodominant.

FPAG16-related antigen in mature parasite

To identify the native *P. falciparum* protein corresponding to FPAG16, the FC27 isolate of *P. falciparum* was biosynthetically labelled with ³⁵S-methionine and labelled proteins of infected cells were immunoprecipitated with anti-FPAG16 sera.

Fig. 1 Analysis and purification of the fused polypeptide FPAG16. 1-ml cultures of Ag16 and λ Amp3 were grown to mid-logarithmic phase, heat-induced at 45 °C for 15 min and incubated at 37 °C for 2 h. The bacterial cells were pelleted and then lysed in 400 μ l of SDS sample buffer (15% glycerol, 4% SDS, 5% β -mercaptoethanol and 62 mM Tris pH 6.8). Forty microlitre samples of each were electrophoresed on a 7.5% SDS-polyacrylamide gel, electroblotted to nitrocellulose and processed according to the method of Burnette¹⁸. The nitrocellulose filter was divided in two and reacted with rabbit anti- β -galactosidase (a) or human anti-*P. falciparum* antibodies (b). In both cases Ag16 is in track 1 and λ Amp3 is in track 2. The sera had been previously depleted of antibodies to *E. coli* by incubation with a 1-ml sonicate of *E. coli* RY1082 for 1 h at 4 °C. After centrifugation at 10,000g for 10 min the supernatant was diluted 1,000-fold with 10 mM Tris, 150 mM NaCl, 3% bovine serum albumin (BSA) pH 7.4. The nitrocellulose sheets were washed and reacted with ¹²⁵I-labelled protein A, rewashed and autoradiographed. c, A Coomassie blue-stained 10% SDS polyacrylamide gel. Tracks 1 and 2 represent the total protein pattern of a lysate of induced λ Amp3 and Ag16 respectively. The major band in track 1 at MW 116,000 is β -galactosidase while the band at MW 146,000 in track 2 is FPAG16. Track 3 shows an aliquot from a 1-l induced culture of FPAG16 that had been processed as follows. The bacterial cells were collected by centrifugation and lysed by treatment with 0.25 mg ml⁻¹ lysozyme, 10 mM Tris pH 8, 2 mM EDTA and 50 mM NaCl. Triton X-100 was added to a final concentration of 0.2% and the solution was made to 10 mM MgCl₂ and 1 μ g ml⁻¹ DNase. After incubation for 30 min at room temperature, cell debris was removed by centrifugation at 850g. Insoluble bacterial proteins were collected by centrifugation at 160,000g, then solubilized in 0.1 M phosphate buffer pH 6.4, 2% SDS and 10 mM dithiothreitol. Track 4 shows an aliquot of a fraction collected after gel filtration of the 'insoluble' proteins on a Sephadex CL 6B column equilibrated with 0.2 M phosphate buffer pH 6.4, 0.1% SDS and 1 mM dithiothreitol. Fractions were analysed by polyacrylamide gel electrophoresis and those containing the purified fusion protein were pooled, desalted on Sephadex G-25, equilibrated with 1 mM β -mercaptoethanol and lyophilized.

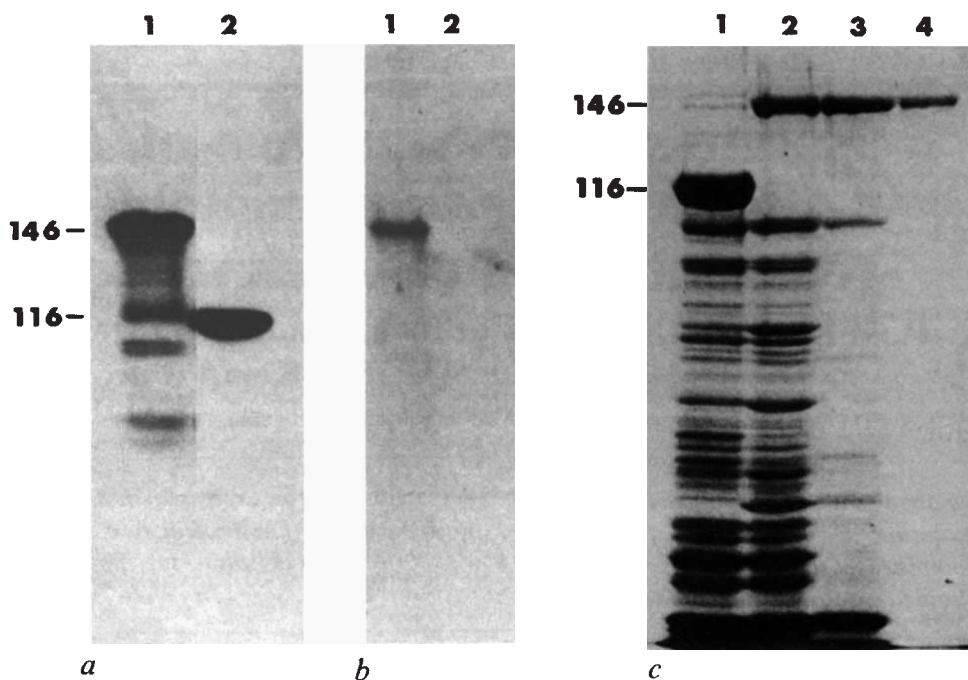
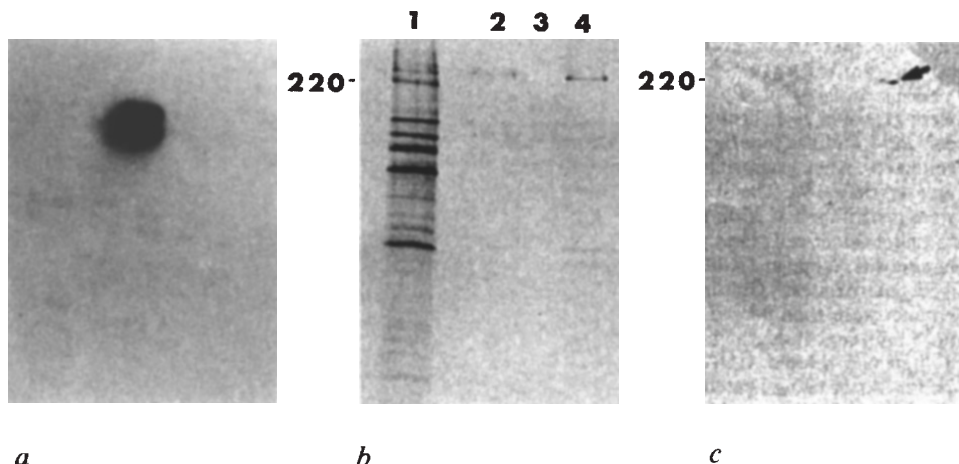


Fig. 2 Specificity of antibodies to FPAg16 and identification of the native protein. **a**, Seventy-eight antigen-positive clones including Ag16 were picked onto a nitrocellulose filter grown at 30 °C until visible colonies were seen, then at 42 °C for 2 h. The colonies were lysed in 1% SDS in chloroform vapour then the filter was washed in 10 mM Tris, 150 mM NaCl, 3% BSA pH 7.4. Rabbit anti-FPAg16 antiserum was absorbed against a sonicate of *E. coli* strain RY 1082 then reacted with the filter at a dilution of 1:1,000. After washing in 10 mM Tris, 150 mM NaCl, 0.05% Tween 20, the filter was reacted with ¹²⁵I-protein A, rewash then autoradiographed for 25 min. The antiserum reacted only with clone Ag16 seen as two rows of triplicate colonies. **b**, **c**, One- and two-dimensional SDS-polyacrylamide gels of immunoprecipitates. Parasites of the FC27 line were grown in asynchronous *in vitro* culture according to the method of Trager and Jensen¹⁹. At a parasitaemia of approximately 3% the parasites were transferred to a methionine-free medium supplemented with ³⁵S-methionine and biosynthetically labelled for 18 h. Parasites were collected by centrifugation and lysed in 0.5% Triton X-100, 150 mM NaCl, 5 mM EDTA, 50 mM Tris pH8. Aliquots of labelled parasite lysate were reacted with various sera. Formalin-fixed *Staphylococcus aureus* was used as a solid-phase reagent. Immunoprecipitates were prepared for one- and two-dimensional SDS-polyacrylamide gel electrophoresis²⁰. Gels were fluorographed then autoradiographed. **b**, Track 1, human anti-*P. falciparum* antiserum; track 2, mouse anti-FPAg16; track 3, serum taken from rabbit prior to immunization with purified FPAg16; track 4, rabbit anti-FPAg16. **c**, A two-dimensional polyacrylamide gel using mouse anti-Ag16. The precipitated protein complex is arrowed. Rabbit anti-FPAg16 antiserum recognized the same set of proteins. Approximate molecular weights are indicated.



Immunoprecipitates were then analysed by one- and two-dimensional gel electrophoresis. There are few leukocytes or reticulocytes in the stored erythrocytes used for parasite culture so that non-parasite-directed biosynthesis of proteins is essentially zero. The gels show that anti-FPAg16 antibodies were directed against a protein with apparent MW 220,000 and an acidic *pI* of 4.2 (Fig. 2). This protein was also found in the culture supernatant of biosynthetically labelled parasites. Reaction of antigens released into culture supernatant with anti-FPAg16 antibodies identified the 220,000-MW form of the protein and 215,000-MW second protein (data not shown). This may represent a cleavage product produced during release of the protein. Antisera raised against λ Amp3 lysogens or purified β -galactosidase did not specifically immunoprecipitate any labelled proteins (data not shown).

Antibodies to FPAg16 were used in immunofluorescence assays to determine the cellular localization of the 220,000-MW protein in the homologous FC27 isolate. Anti-FPAg16 reacted with the mature trophozoite and more intensely with the schizont stage (Fig. 3). It did not react with immature ring forms. A characteristic feature was that of a brightly staining schizont, apparently bathed in fluorescent material and often surrounded by an extracellular zone of speckle fluorescence composed of discrete small particles (Fig. 3e). Fluorescence of the surface of infected erythrocytes was not seen when the assay was performed using unfixed cells. When *P. falciparum* isolates from Thailand and Ghana (isolates K1 and NF7 respectively, both supplied by Dr D. Walliker) were examined with the same sera, no fluorescence was detected. This suggested that the 220,000-MW antigen was isolate-specific, and that any analogous protein in the other two isolates must be immunologically distinct. We conclude that Ag16 encodes a major mature-stage antigen that varies among isolates.

Clone Ag16 corresponds to an S-antigen

The isolate specificity and molecular characteristics described above for the *P. falciparum* protein corresponding to FPAg16 were strikingly similar to those recently described for the S-antigen of FC27¹⁰. The possibility that Ag16 encodes the S-antigen of FC27 could be readily tested because of the availability of a cloned line of *P. falciparum* expressing a variant S-antigen. This cloned line, E12, was derived from isolate FC27 by limit dilution culture and has an S-antigen antigenically

similar but MW 30,000 greater than the antigen in FC27¹⁰. Conclusive evidence that the antigen expressed in clone Ag16 was part of the S-antigen was obtained from immunoblotting experiments in which antigens in culture supernatants of E12 and FC27 were probed with the rabbit and mouse antisera raised against FPAg16. As shown (Fig. 4), these antisera detected a heat-stable antigen in the supernatants of FC27 and E12 with apparent MW of 220,000 and 250,000 respectively. The pattern of antigens detected in the two supernatants with the antisera to FPAg16 was identical to that detected with a human serum having a high titre of antibodies to S-antigen. Isolate K1 from Thailand and isolate NF7 from Ghana produce S-antigens antigenically distinct from that of FC27 (unpublished observations).

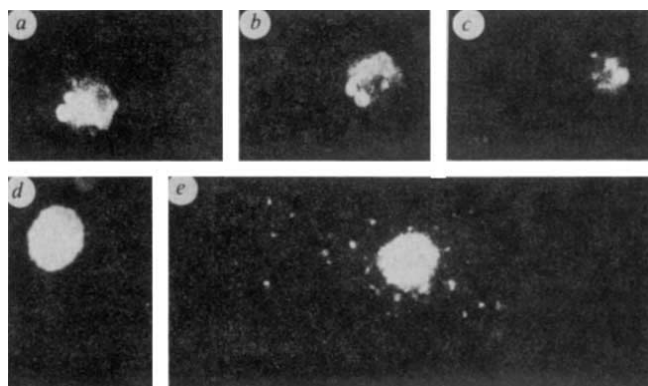
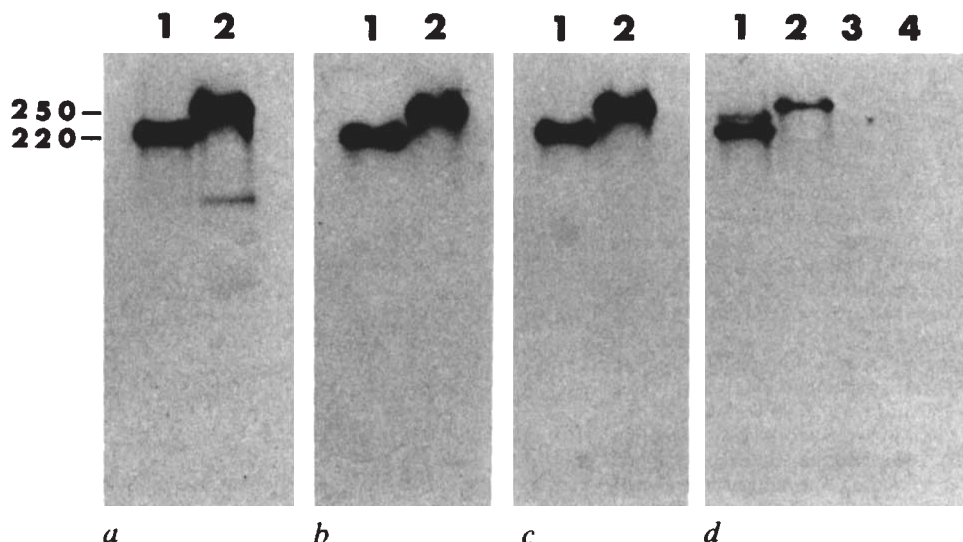


Fig. 3 Indirect immunofluorescence of *P. falciparum* reacted with anti-FPAg16 antibodies. The FC27 isolate of *P. falciparum* was synchronized by treatment with 5% sorbitol according to the method of Lambros and Vanderberg²¹ then grown *in vitro*¹⁹. Thin smears were taken and fixed by immersion in 90% acetone, 10% methanol. The parasites were then reacted with a 1:500 dilution of mouse anti-FPAg16 antibodies for 30 min at room temperature. After washing in human tonicity phosphate-buffered saline, fluorescein-conjugated anti-mouse IgG antiserum was added. After washing and immersion in glycerol the slides were examined under UV light. Duplicate smears were stained in 10% Giemsa, 10% methanol to determine the stage of development of the parasites. Panels a–c, pigmented parasites presumed to be late trophozoites or early schizonts. d, e, Fully mature schizonts. Similar fluorescence patterns were obtained with rabbit anti-FPAg16 sera.

Fig. 4 Immunoblot analysis of culture supernatants of *P. falciparum* using anti-FPAG16 antibodies. Various isolates of *P. falciparum* were grown in asynchronous *in vitro* culture¹⁹. Culture supernatants were collected when parasitaemia was greater than 1%. The supernatants were centrifuged at 350g for 10 min to pellet intact cells then the supernatant was respun at 1,400g to pellet free merozoites. Culture supernatants were then heated at 100 °C for 5 min, centrifuged at 15,000g for 10 min and diluted 1:10 in sample buffer. After being heated for 2 min at 100 °C, 40 µl were added to each lane of a 7.5% polyacrylamide SDS gel. After electrophoresis the gels were electroblotted to nitrocellulose and processed according to Burnette¹⁸. Filters were reacted with a variety of antisera. *a-c*, Track 1, FC27 supernatant; track 2, E12 supernatant; *d*, track 1, FC27 supernatant; track 2, E12 supernatant; track 3, K1 supernatant, a Thai isolate; track 4, NF7, a Ghanaian isolate. The sera used to probe the filters were: *a*, 1:500 dilution of a human serum previously characterized as having a high titre directed against the S-antigen of FC27¹⁰; *b*, *d*, 1:1,000 dilution of mouse anti-FPAG16 serum. *c*, 1:1,000 dilution of rabbit anti-FPAG16 antibodies. The doublet in track 1 of *d* is seen regularly and is similar to that found by immunoprecipitation of culture supernatant. Approximate molecular weights ($\times 10^{-3}$) are indicated.



When culture supernatants of these isolates were probed with mouse anti-Ag16, no S-antigen was detected. Thus these two non-Papua New Guinea lines have S-antigens lacking any antigenic cross-reactivity with FPAG16 (Fig. 4d).

Nucleotide and amino acid sequence of Ag16

DNA from Ag16 was purified and the *P. falciparum* cDNA 763-bp segment released by digestion with *EcoRI* and subcloned into the *EcoRI* site of bacteriophage M13 sequencing vector Mp9¹³. Recombinant Mp9 phage DNA was isolated and hybridized to the original clone Ag16 to confirm that it contained the correct cDNA (data not shown). Dideoxy sequencing was performed on Mp9 clones containing the cDNA insert in both orientations.

The entire cDNA was composed of a 33-bp sequence repeated 23 times (Fig. 5). There is a recognition site for the restriction enzyme *Sau3A* within the 33-bp repeat and mapping with this enzyme confirmed the presence of this site throughout the cDNA sequence (data not shown). Although the 33-bp unit is not repeated precisely, the variation is strictly limited, occurring only in the third base of some codons. Thus, because of the degeneracy of the genetic code the amino acids are unchanged. The variation in the third base is limited to only 4 of the 11 amino acids (Fig. 5). All alanine codons at position 2 of the repeat are strictly conserved while the alanine codons at position 4 vary in the third nucleotide. Similarly, the glycine codons at position 8 are conserved while those of position 7 vary. Hence the 11-amino acid sequence -Pro-Ala-Lys-Ala-Ser-Gln-Gly-Gly-Leu-Glu-Asp- is tandemly repeated throughout this segment of the FC27 S-antigen. A synthetic peptide of this sequence binds antibodies to the S-antigen of FC27 (R.F.A., unpublished observation) confirming that this is the correct reading frame. The cDNA covers about 15% of the sequence of the total protein and encodes 23 repeats. It is likely, however, that the repeat is distributed throughout most of the molecule because we have observed a distinctive 'ladder' pattern of proteolytic breakdown products of native S-antigen in immunoblotting experiments (data not shown). The observation that S-antigen can be biosynthetically labelled using methionine, an amino acid not found in the repeat, suggests that a portion of the native molecule is not composed of the 11-amino acid repeat structure.

The structure of the S-antigen bears a remarkable resemblance to that recently described for the surface coat protein of the sporozoite of *P. knowlesi*¹⁴. The sporozoite protein con-

Glu	Phe	Arg	Pro	Ala	Lys	Ala	Ser	Gln	Gly	Gly	Leu	Glu	Asp
GAA	TTC	CGT	CCC	GCA	AAG	GCT	AGT	CAA	GGA	GGA	TTA	GAA	GAT
LINKER													
			2	T		A	T		A				
			3	C		G	T		A				
			4	T		A	T		A				
			5	T		A	T		T				
			6	T		A	T		A				
			7	T		A	T		T				
			8	T		G	A		A				
			9	T		A	T		T				
			10	T		A	T		A				
			11	T		A	T		T				
			12	T		A	T		T				
			13	C		G	T		T				
			14	T		A	T		T				
			15	C		G	T		A				
			16	T		A	T		A				
			17	T		A	T		T				
			18	T		A	A		A				
			19	T		A	A		A				
			20	T		G	A		A				
			21	T		A	A		A				
			22	T		A	A		T				
			23	CCT	GCA	AAA	GCT	AGT	CAA	GGA	GGA	TTA	GAA
													CCT
													CGG
													AAT
													TC
													LINKER

Fig. 5 Nucleotide sequence of the Ag16 cDNA. The dideoxy chain termination method²² was used for sequence determination. The Ag16 *EcoRI* cDNA fragment was subcloned into M13mp9 in both orientations and sequenced. The number of repeat units was confirmed by partial restriction mapping with *Sau3A* and *MboII*. The first and last repeat units are shown in full, but only the base changes occurring in the third base of certain codons are indicated in the other repeats. The amino acids are identical for each repeat and are thus shown only for the first repeat unit. The first two amino acids (Glu, Phe) are encoded by the *EcoRI* linker as are the C and G of the Arg codon. Pro has been arbitrarily designated the first amino acid of the repeat unit. The reading frame is shown in phase with β -galactosidase.

tained 12 repeats of a 12-amino acid sequence that together accounted for one-third of the total amino acid sequence of the protein.

Organization of S-antigen genes

DNA from four *P. falciparum* isolates, FC27 and E12 from Papua New Guinea, K1 from Thailand and NF7 from Ghana, was digested with restriction enzymes and size-fractionated by agarose gel electrophoresis. The DNA was transferred to nitrocellulose and probed with ³²P-labelled Ag16 cDNA. As expected, the probe hybridized to DNA from FC27 and E12 (Fig. 6), both of which express the native antigen, and did not hybridize to human DNA (data not shown). Surprisingly there was no hybridization to either K1 or NF7, the non-expressing lines (Fig. 6). This was true even in conditions of lowered stringency (twice standard citrate saline) when moderately homologous sequences would hybridize. This same filter was subsequently reprobbed with an unrelated cDNA sequence and signals were seen in all tracks (data not shown) demonstrating that the failure of Ag16 to hybridize in some tracks was not a technical artefact.

The *Eco*RI fragment sizes of FC27 and E12 DNA were consistent with the immunoblot analysis. The S-antigen gene of E12 is about 1 kilobase (kb) larger than that of FC27, just large enough to encode the extra 30,000-MW protein sequence. It is most likely that the gene in E12 arose from duplication of the repetitive part of the FC27 gene. It is not clear if this occurred during long-term *in vitro* culture, during selection of clonal populations by limit-dilution culture, or before the isolate was established in culture.

The apparent absence of the FC27 S-antigen gene from the two non-Papua New Guinea strains was unexpected. A number of very different mechanisms could account for this. For example, the entire repertoire of S-antigens may be represented in every isolate by one or a few repeat units. Such 'minigenes' may not be detectable by Southern hybridization in the conditions used. Alternatively, if the gene is truly absent and if the different S-antigen genes are not allelic then there may be some mechanism for generation of diverse antigen genes within a particular parasite line, possibly during sexual recombination in the mosquito.

Conclusions

The data show that clone Ag16 encodes a portion of the S-antigen of strain FC27. A substantial part of the protein consists of tandem repeats of 11 amino acids apparently strictly conserved within the protein. In contrast those isolates that do not produce this S-antigen apparently lack genes of detectable homology.

The structure of the S-antigen of FC27 is like the surface coat of the *P. knowlesi* sporozoite in that both have tandemly repeating regions: 11 amino acids in the S-antigen and 12 amino acids in the sporozoite coat protein¹⁴. However, the two sequences are not homologous. A striking difference is that serological variation of sporozoite coat antigens within one species is very limited^{15,16} whereas the S-antigen varies between strains, giving rise to many non-cross-reacting 'serotypes'. Studies with monoclonal antibodies have shown that the sporozoite surface coats of *Plasmodium vivax* and *P. falciparum* also contain a repeating subunit structure, which must be distinct for each species because of the differing immunological reactivities^{15,17}. This paradox of precisely conserved structural units repeating many times, with widely differing antigenic sequences, may be a feature of *Plasmodium* antigens.

The diversity of S-antigens suggests that they are subject to strong selective pressures. The host immune system is the most obvious source of selection, suggesting that the immune response to this antigen must be of some protective value to the host. Conversely, the extreme variability of the S-antigen would be of benefit to the parasite. The variable S-antigen is present in

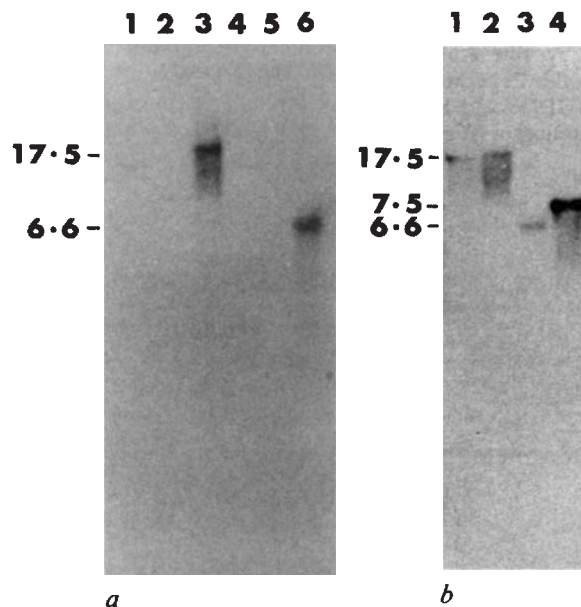


Fig. 6 Southern blot analysis of genomic DNA of *P. falciparum* by Ag16 cDNA. *P. falciparum* isolates grown in *in vitro* culture were collected and concentrated by lysis with 0.15% saponin solutions¹¹. The cell pellets were dissolved in 6 M guanidine hydrochloride, 0.4 M Na acetate pH 5.2. The DNA was twice banded in CsCl gradients then dialysed against 10 mM Tris, pH 8, 1 mM EDTA. Aliquots (3 µg) were restricted with either *Eco*RI or *Hind*III and the digestion products electrophoresed in a 1% agarose gel. The DNA was transferred to nitrocellulose²³ and the filters were baked *in vacuo* then prehybridized in 5× standard salt citrate (SSC), 50% formamide, 50 µg ml⁻¹ salmon sperm DNA, 10 µg ml⁻¹ poly(C) and IX Denhardt's solution. Purified Ag16 cDNA was labelled by nick-translation and hybridized to the filters at 10⁶ c.p.m. ml⁻¹ at 42 °C. The filters were washed in 2×SSC at 65 °C then autoradiographed. *a*, 1–3, *Hind*III digest; 4–6, *Eco*RI digests; 1, 4, NF7 DNA; 2, 5, K1 DNA; 3, 6, FC27 DNA. *b*, 1, 2, *Hind*III digests; 3, 4, *Eco*RI digests; 1, 3, FC27 DNA; 2, 4, E12 DNA.

mature blood stages of *P. falciparum* in close association with the merozoite. If there are indeed many different S-antigen sequences, then many attacks of malaria may be required to sensitize the immune system against all of the sequences. The number of different S-antigen sequences is not yet clear, for example, FC27 and E12 differ in size but contain the same or a closely related repeat whereas K1 and NF7 must contain (a) different sequence(s). The presence of S-antigen in the serum of an infected individual may be utilized for purposes of 'immune evasion' by the parasite. Circulating S-antigen bearing a repeating epitope should absorb serum antibody, and possibly reduce the availability of antibodies against late parasite stages.

We postulate that the S-antigens of *P. falciparum* are a family of molecules comprised of repeating, antigenically-distinct subunits. It is unlikely that the repeating portion of the S-antigen has a functional role in parasite development as it varies so greatly among isolates. However, it may be attached to or in close association with some other important molecule, so that a highly variable S-antigen could camouflage this second molecule from the immune system. Alternatively, S-antigens because of their repeating structure could directly stimulate B-cells with resultant exhaustive differentiation. While the exact role of S-antigens remains to be determined, our observation here that they must differ substantially in sequence in different strains of *P. falciparum* raises the possibility that this sequence variation allows evasion of the human immune system.

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Activation of a *Qa/Tla* class I major histocompatibility antigen gene is a general feature of oncogenesis in the mouse

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A cDNA clone corresponding to a mRNA present at elevated levels in transformed fibroblasts encodes a Qa/Tla class I major histocompatibility complex (MHC) antigen. High levels of this mRNA are found in all tumour cells tested; the transcript can undergo alternative splicing; and a repetitive sequence within the transcription unit has the characteristics of a transposable element. The immunological implications of MHC gene activation in tumour cells are discussed.

WE HAVE recently described the isolation of a number of cDNA clones which correspond to mRNAs present at higher levels in the simian virus 40 (SV40)-transformed mouse cell line SV3T3 Cl38 than in the normal parental line BALB/c 3T3¹. These clones were divided into five sets on the basis of restriction enzyme mapping and cross-hybridization experiments^{1,2}. Clones of a given set are thus derived from the same, or closely related, mRNAs. The prototype cDNA clone of Set 1, pAG64, contains a dispersed repetitive element present thousands of times in the mouse genome, and hybridizes to three SV3T3 Cl38 mRNAs, of sizes 1.6 kilobases (kb), 0.7 kb and 0.6 kb¹.

Elevated levels of the mRNAs defined by Set 1 are a general feature of mouse fibroblasts transformed not only by SV40 but also by polyoma virus (another DNA tumour virus), by the retroviruses Rous sarcoma virus and Abelson murine leukaemia virus (A-MuLV), and by the chemical carcinogens methylcholanthrene and methylcholanthrene epoxide¹. Elevated levels of the 1.6-kb mRNA are a universal feature of transformed mouse fibroblasts, whereas the 0.7-kb and 0.6-kb mRNAs are elevated in many but not all lines, suggesting that they derive from a transcription unit(s) which can be regulated independently of that encoding the 1.6-kb mRNA¹.

Expression in non-fibroblast cells

We have now asked whether high levels of the mRNAs defined by Set 1 are found in mouse transformed and tumour cell lines of non-fibroblast origin. Figure 1 shows that this is the case for an AKR thymoma cell line, a mastocytoma cell line, an A-MuLV-induced B-cell tumour line, a Friend erythroleukaemia cell line, a myeloma cell line, a neuroblastoma cell line and a rat hepatoma cell line. It thus appears that activation of the gene(s) defined by Set 1 is a general feature of oncogenesis in

the mouse. We have also analysed in some detail the expression in normal cells and tissues of mRNAs that hybridize to Set 1 cDNA probes. Very low levels of the 1.6-kb mRNA are found in normal established cell lines such as BALB/c 3T3 A31 and BALB/c N¹, but we have not detected the two smaller mRNAs in these lines. As shown in Fig. 1, significant levels of the 1.6-kb mRNA are found in normal thymus, which is the only adult organ in which we have clearly detected this transcript. This RNA cannot be detected in adult spleen cells, but when such cells are stimulated with phytohaemagglutinin there is a clear induction not only of the 1.6-kb mRNA but also of an ~1.1-kb transcript of unknown identity which we have not observed elsewhere.

A shared repetitive element

The presence of a dispersed repetitive element within pAG64 raises the question of the relationship between this element and the three Set 1 mRNAs found in transformed cells. We have therefore constructed subclones of pAG64 (Fig. 2c) that contain either predominantly the repetitive element (pAG64-E) or exclusively non-repetitive sequences (pAG64-C), and have used these as probes in transfer hybridization experiments. Figure 2a shows that pAG64-E detects all three Set 1 mRNAs, whereas, as shown in Fig. 2b, pAG64-C detects only the 1.6-kb mRNA. These data show that the three Set 1 mRNAs hybridize to pAG64 because each contains the repetitive element, and that pAG64 is a cDNA copy of the 1.6 kb mRNA.

pAG64 encodes a *Qa/Tla* antigen

The complete nucleotide sequence of pAG64 has been determined, and is given in Fig. 3. To our considerable surprise, comparison of this sequence with known nucleotide sequences

indicates that pAG64 is a cDNA copy of a mRNA encoding a class I major histocompatibility complex (MHC) antigen. Indeed, the published sequence of a cDNA clone, pH-2^d-1, which has been assigned as an *H*-2-like gene of the *d* haplotype, is almost identical to that of pAG64 (Fig. 4)³. The non-repetitive region of pAG64 has a sequence homologous to all those published for MHC antigens, but the Set 1 repetitive element is found in only some MHC mRNAs. It is absent from a clone assigned as encoding the *H*-2K^d (ref. 3) antigen, and from two *H*-2K^b sequences^{4,5}. At least a part of the repetitive element is present in a third clone described by Lalanne *et al.*³, and in *H*-2D^b and *H*-2L^d sequences^{6,7}. In these cases the published sequences do not extend to the 3' end of the mRNA, and it seems likely that the remainder of the repetitive element and the additional 3' non-coding sequences are present in the corresponding mRNAs.

BALB/c mice are of the *H*-2^d haplotype. We have therefore compared the sequence of pAG64 with the published sequences of *H*-2K^d (ref. 3), *H*-2L^d (ref. 7) and *H*-2D^d (ref. 8). In each case there are multiple, clustered differences: approximately 10% between pAG64 and both *H*-2K^d and *H*-2L^d, and approximately 7% between pAG64 and *H*-2D^d (only 291 base pairs (bp) of overlapping sequence are available for this comparison). As there are single genes encoding the *H*-2 K, L and D antigens, pAG64 cannot be derived from a classical *H*-2 gene and is likely to define one of the genes of the *Qa/Tla* complex. Indeed, hybridization with pAG64 at reduced stringency allows detection of the slightly smaller *H*-2K transcript in BALB/c 3T3 cells (K.-H. Westphal and P. W. J. R., unpublished data). pAG64 is more than 99% homologous to the cDNA clone pH-2^d-1, which represents a mRNA that was isolated from a lymphoid tumour (SL2) that was grown as ascites in DBA/2 mice. SL2 tumour cells express *H*-2K^d, *H*-2L^d, *H*-2D^d and *Tla* (ref. 3) antigens, and so it is likely that pH-2^d-1 also defines a *Qa/Tla* gene. Our data on the expression of mRNA homologous to pAG64 in embryonic cells also argue strongly against this clone being derived from a classical *H*-2 gene (see below).

Characteristics of repetitive elements

As shown in Fig. 3, the Set 1 repetitive element interrupts two sequences which are contiguous in *H*-2K genes. Furthermore, the Set 1 element is flanked by a 12-bp direct repeat, only one copy of which is present in *H*-2K genes. This suggests that the presence of the Set 1 repetitive element in the 3' untranslated region of some, but not all, MHC genes reflects transposition events during the evolutionary history of the MHC. In support of the idea that the Set 1 repetitive element is mobile in the genome, Steinmetz *et al.* have characterized a genomic clone mapping to the *Qa* region of the MHC, which has no Set 1 repeat in what would be the 3' untranslated region of the mRNA but rather contains a copy of the repeat, in the opposite orientation, within one of the introns⁹.

Fig. 2 Transfer hybridization analysis of Set 1 transcripts using repetitive and non-repetitive probes. 1- μ g aliquots of polyadenylated, cytoplasmic RNA were fractionated by electrophoresis in a 1% (w/v) agarose gel containing formaldehyde. In *a*, *b*, lanes 1 contain RNA from adult BALB/c mouse liver and lanes 2 RNA from SV3T3 Cl36. In *a* the probe was pAG64-E, a subclone of pAG64 containing predominantly the repetitive element (nucleotides 1,099–1,285 in the sequence of pAG64). In *b* the probe was pAG64-C, a subclone of pAG64 containing unique coding sequence (nucleotides 580–1,098 in the sequence of pAG64). *c* Shows the structure of pAG64. The Set 1 repetitive element is indicated by the boxed region. The restriction fragments used for the construction of subclones are indicated.

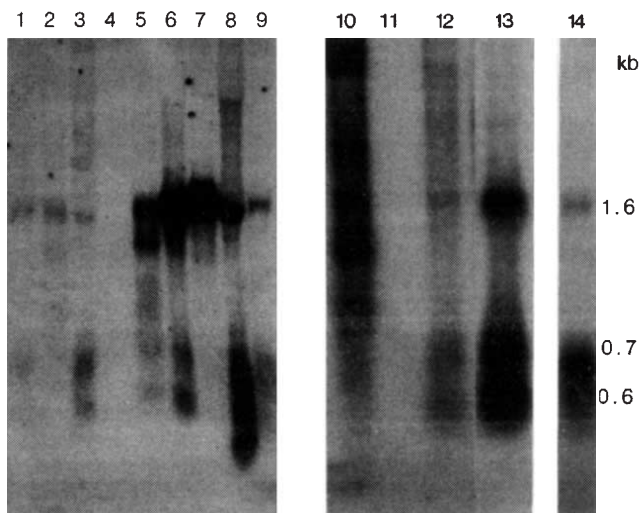
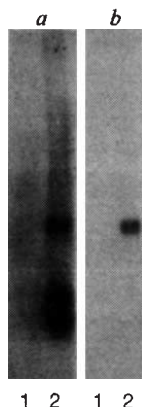
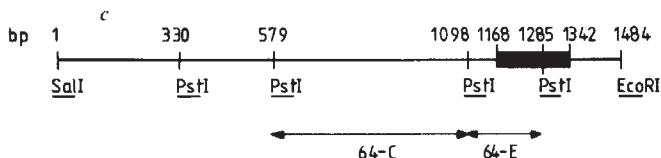


Fig. 1 Transfer hybridization analysis of Set 1 transcripts in transformed and tumour cell lines of non-fibroblast origin. 10- μ g aliquots of polyadenylated, cytoplasmic RNA were fractionated by electrophoresis in a 1% (w/v) agarose gel containing formaldehyde, transferred to filters and hybridized with ³²P-labelled pAG64. The RNA samples were taken from: lane 1, SV3T3 Cl38 cells; lane 2, adult BALB/c mouse thymus; lane 3, AKR thymoma cell line BW5147 C1A11⁴⁴; lane 4, adult BALB/c mouse spleen; lane 5, phytohaemagglutinin (PHA)-stimulated adult BALB/c mouse spleen cells; lane 6, mastocytoma cell line P815⁴⁵; lane 7, A-MuLV-induced B-cell tumour line (E. S. Lennox, personal communication); lane 8, Friend erythroleukaemia cell line GM0086D, from DBA/2 mice (obtained from the NIGMS Human Genetic Mutant Cell Repository); lane 9, myeloma cell line SP2/OAG14⁴⁶; lane 10, DNA size markers; lane 11, blank; lane 12, rat hepatoma line FAZA⁴⁷; lane 13, P815 cells; lane 14, neuroblastoma cell line PLATT (B. Hogan, personal communication).

Methods: The procedures for quantitating polyadenylated, cytoplasmic RNA by hybridization to ³H-poly(rU) and for gel electrophoresis and transfer hybridization have been described previously^{1,15}. PHA-stimulated spleen cells were prepared as follows: whole BALB/c spleen cell suspensions were adjusted to 10⁶ leukocytes ml⁻¹ in RPMI 1640 supplemented with 10% (v/v) fetal calf serum, 2 × 10⁻⁵ M 2-mercaptoethanol and 50 μ g ml⁻¹ PHA (*Phaseolus vulgaris* lectin, Sigma), and cells were collected after incubation at 37 °C for 72 h.

The Set 1 repetitive element is a member of the B2 family originally defined by hybridization to 'fold-back' nuclear RNA¹⁰. The copy of the B2 repeat in pAG64 diverges considerably (~16%) from the consensus B2 family sequence¹⁰, and this divergence, together with the fact that we have always performed our hybridizations in conditions of high stringency, could account for our not detecting other B2-containing mRNAs, such as the 2.0-kb species present in mouse liver¹¹, when using the Set 1 repetitive element as a probe.

The 0.7-kb and 0.6-kb mRNAs that hybridize to the Set 1 repetitive element are not dissimilar in size to the mRNAs¹² that encode β_2 -microglobulin, the non-polymorphic protein found associated with class I antigens. However, the nucleotide sequence of β_2 -microglobulin mRNA¹³ shows no homology to the Set 1 repetitive element.



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AGA	TAT	GAG	10	CGG	GCG	20	TGG	ATG	30	CAG	CAG	GAG	40	GAG	TAT	50	GAG	CUG	60	ACA	CGC	AGA	70	AGC	AGC	80	AAT	GAG	CAG	AGT	90
ATC	CGA	GTC	100	GAC	CTC	AGG	110	CGC	CTC	GGC	TAC	TAC	130	AGC	GCG	140	GCG	TCT	150	CTA	CTG	160	ATG	GCT	170	GCG	TGT	CAG	GTG	180	
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CTG	ACC	CAG	550	GAA	ATG	GAG	CTT	GTC	570	GAG	ACG	GCT	580	GAT	GGA	590	CTC	CAG	600	TGG	GCA	TCT	610	GTC	620	CTT	GGG	AGA	630		
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AGA	AAC	ACA	820	GGA	AAA	GGA	GGG	CAG	840	GCT	CTC	GCT	850	CG	CTC	860	ACC	CTC	870	GAT	ATC	CTC	CTC	CCA	GAT	TCT	AAA	G	861		
CAG	TGCT	CTG	870	TGCT	TGCT	TGCT	TGCT	TGCT	880	TGCT	TGCT	TGCT	890	TGCT	TGCT	900	TGCT	TGCT	910	TGCT	TGCT	920	TGCT	TGCT	930	TGCT	TGCT	TGCT	TGCT	940	
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Altered nucleotide sequences of a translocated *c-myc* gene in Burkitt lymphoma

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The nucleotide sequence of a translocated c-myc gene in a Burkitt lymphoma reveals multiple base changes in the coding region. Twenty-five base changes, generating 16 codon alterations, were found in the first coding exon; no changes occur in the second coding exon. These changes are probably the result of somatic mutations that occurred during and after translocation, and may contribute to oncogenesis by allowing synthesis of an altered c-myc gene product.

BURKITT lymphoma is a malignancy of human B lymphocytes characterized by the presence of chromosomal translocations involving a segment of chromosome 8 (8q24→ter)^{1,2} with one of the three chromosomes containing the immunoglobulin loci (14, 2 or 22)^{3–6}. The significance of the correlation between this consistent breakage of chromosome 8 and the transformation process was revealed by the observation that a cellular homologue of the avian myelocytomatosis retrovirus transforming gene, the *c-myc* gene, is located at the position of the cytogenetic breakpoint in Burkitt lymphoma^{7–9}. Furthermore rearrangement of the *c-myc* gene was observed in a proportion of Burkitt lymphoma cell lines^{7,9}. Recently we described a translocated *c-myc* gene from Raji cells (t8; 14) in which immunoglobulin *C_γ1* and *c-myc* genes had been joined by translocation¹⁰. These and other studies showed that the human *c-myc* gene is composed of three exons, of which the first is apparently non-coding¹¹ whilst the second and third exons are homologous to the *v-myc* gene, the methionine initiation codon occurring near the start of exon 2. In the case of the rearranged *c-myc-C_γ1* gene segment from Raji cells the translocation breakpoint was a short, undefined distance upstream of the first exon¹⁰. In some other cases the break occurs a great distance from the *c-myc* gene, whilst in others it actually occurs within the gene^{12,13}.

The variability in chromosomal breakpoint positions seen in Burkitt lymphoma presents a paradox for the effect of the translocation on the *c-myc* gene. Translocation points that occur far upstream of the first exon of *c-myc* do not affect the exon structure of this gene. The possibility that an enhanced level of expression of *c-myc* resulting from the translocation of *c-myc* into the immunoglobulin locus is responsible for the tumorigenic effect does not seem to be the complete answer. First, high levels of *c-myc* expression can be observed in non-Burkitt lymphoma B-cell lines¹⁰ and other tissues¹⁴. Second, an immunoglobulin transcriptional enhancer present near the *J_H-S_μ* region is removed in some cases from the *c-myc* gene by the translocation event (see this issue¹⁵).

A different hypothesis for the role of the translocated *c-myc* allele in Burkitt lymphoma is the possibility of the acquisition of somatic mutations in the translocated *c-myc* gene, in a manner

similar to the immunoglobulin variable region genes, by virtue of its presence within one of the three immunoglobulin loci. We now present evidence for such somatic mutation in a translocated *c-myc* gene. The complete nucleotide sequence of the translocated *c-myc* gene from Raji cells is given and this sequence shows 16 codon changes from the normal *c-myc* counterpart exclusively occurring within exon 2.

Translocated *c-myc* gene organization

The structure of the translocated *c-myc* gene from Raji cells has been investigated by detailed restriction mapping and nucleotide sequencing. Figure 1 shows the restriction map of this translocated *c-myc* gene and of the *C_γ1* gene associated with it. The position of the chromosomal breakpoint has been located to within 1 kilobases (kb) of the *Cl**a**I* site found in the subclones RB19 XS1 and RB6 XH6 since the *H**ind**III* site of the normal *c-myc* gene (seen at the limit of the RB6 XH6 clone) is absent from the translocated gene. The breakpoint thus occurs about 2 kb upstream (5') of the *X**ho**I* site within exon 1 of the *c-myc* gene. At the junction of this breakpoint, there is a segment of about 1.2 kb which is homologous to the switch (*S*) sequence of the *C_μ* gene (the region immediately upstream of the *C_μ* gene which is involved in the switch recombination process). The *S_μ* sequence abuts a 1.8 kb segment which contains the *S_γ* region (contained and mapped within clone RB19 HS2). The organization of this complex *S_μ/S_γ* segment near the translocated *c-myc* gene is reminiscent of the homologous region from switched *C_H* genes and suggests that the *c-myc/C_γ* association in Raji DNA may have resulted from a secondary switching process (see below).

The structure of the translocated *c-myc* gene shows the three exon structure of the normal gene (Fig. 1). The *X**ho**I* restriction site within exon 1 is retained in the translocated *c-myc* and the restriction map for 1.5 kb upstream of this site is identical in translocated (clone RB19 XS1) and normal (clone RB6 XH6) *c-myc* genes. Evidently, then, the 5'-end of the rearranged *c-myc* gene is the same as the normal gene. Several nucleotide differences, shown in Fig. 2, however, exist in the region of the first exon (around the *X**ho**I* site) between the translocated and

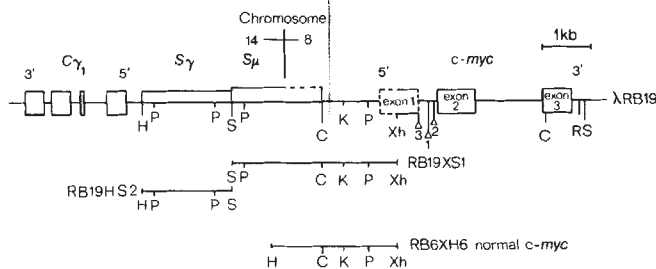


Fig. 1 Structure of the translocated *c-myc* gene locus from Raji cells (t8; 14). The top line illustrates the association of the *c-myc* gene and the *Cγ1* immunoglobulin gene, from Raji cells, in the phage clone *λRB19*¹⁰. The dotted line near the junction of chromosomes 8 and 14 indicates the region to which the breakpoint has been localized. The 5' end of the *c-myc* exon 1 is not known precisely and is also indicated by dashed lines. Clones *RB19XS1*, *RB19HS2* are subclones from *λRB19* in M13 phage using *XhoI* plus *SacI* or *HindIII* plus *SacI* respectively. Clone *RB6XH6* was derived from *λRB6* (the normal counterpart of the *c-myc* gene from Raji cells) by subcloning in M13 phage using *XhoI* plus *HindIII*. H, *HindIII*; P, *PstI*; S, *SacI*; C, *ClaI*; K, *KpnI*; Xh, *XhoI*; R, *EcoRI*.

normal *c-myc* genes including a region of 10 bases deleted from the translocated *c-myc* gene. The possible importance of these differences is at present hard to assess, as the functional significance of the first, apparently non-coding, exon is unknown. Although the promoter site for the *c-myc* gene has not been identified, it is likely to occur within this region as suggested by previous sequencing data¹¹. The 3' end of the first exon in the translocated gene is, however, affected by three deletions found in the intervening sequence between exons 1 and 2 (Figs 1, 3) and identified by comparison with published human *c-myc* sequences. One of these deletions (designated deletion 3) removes the donor splice site suggested to occur at the 3' end of exon 1 on the basis of cDNA sequencing¹¹. However 76 bases from the site of deletion 3, a potential splice donor site occurs (around residue 917 in Fig. 3). This sequence (TTG/GTAAGT) conforms to the consensus sequence formulated by a comparison of published splice signals¹⁶ so could replace the normally used donor site. This splice site would yield an altered exon 1 which is similar in size to that of the normal gene. The putative acceptor site just upstream of the methionine residue is not affected by the deletions in intron 1 of the translocated gene. Finally deletion 1, in the sequence shown in Fig. 3, includes the sequence previously proposed to act as a promoter site in the *c-myc* gene¹⁷ so that this site obviously cannot be active in this cell line.

Codon changes in translocated *c-myc*

Figure 3 shows the nucleotide sequence of the translocated *c-myc* gene and includes the amino acid sequence predicted to

Table 1 Amino acid differences between *c-myc* proteins

Residues	Human		Chicken	
	Normal	Raji	<i>c-myc</i>	<i>v-myc</i>
6	Ser	Thr	Ser	Ser
7	Phe	Ile	Leu	Leu
10	Arg	Lys	Lys	Lys
39	Glu	Asp	Glu	Glu
56a	—	Leu	—	—
58	Thr	Asn	Thr	Met
88	Gly	Asp	—	—
92	Ser	Asn	Cys	Cys
114	Ser	Asn	Ser	Ser
120	Asp	Gly	Asp	Asp
149	Leu	Val	Leu	Leu
171	Cys	Ser	Ser	Ser
203	Ser	Arg	Ala	Ala
230	Ser	Ala	—	—
240	Leu	Phe	—	—
245	Pro	Ser	Pro	Pro

The residue numbers refer to those of the normal human *c-myc* protein deduced from the DNA sequence. Dashes indicate absent residues.

be encoded by the second two exons. Exon 2 consists of 759 base pairs (bp) (253 codons) and exon 3 consists of 562 bp (187 codons) with the two exons being separated by an intervening sequence of 1,373 bp. When compared with the previously published sequences of human *c-myc* genes^{11,17,18} we find a dramatic number of changes in the protein coding portion of the translocated *c-myc* gene. These differences are shown in Fig. 3. There are a total of 25 base changes in exon 2 as well as an insertion of three nucleotides (CTG) contributing an additional leucine residue to protein sequence (at position 1,320 in Fig. 3). These base substitutions include 16 codon alterations within exon 2 which would yield a *c-myc* protein of considerably altered sequence. In addition to these alterations in exon 2, the intron between the two protein coding exons has 9 base differences from the published sequences, but there are no further base substitutions compared with published *c-myc* sequences beyond position 2,562 in our sequence. Thus there are no differences whatever in the third exon of the translocated gene. This interesting clustering of nucleotide alterations gives a total of 6.3% amino acid changes in exon 2.

One potential problem with comparing human protein sequences is that of polymorphic differences. In the case of human *c-myc* there are three published DNA sequences^{11,17,18}, and there are no amino acid substitutions predicted by these sequences. However, two of the sequenced genes were cloned from the same DNA source and so may represent the same gene^{17,18}. To strengthen the data on the conservation of the *c-myc* protein sequence we have determined the sequence of

CTTCCCCACCCCTCCCCACCCCTCCCATAAAGCGCCCTCCCGGGTTCCCAAGCAGAGGGCGTGGGGGAAAGAAAAAGATCCTCTCTCGCTAATCTCCGCCACCGGCCCTTTATAATGC

 CTTCCCCACCCCTCCCCACCCCTCCCATAAAGCGCCCTCCCGGGTTCCCAAGCAGAGGGCGTGGGGGAAAGAAAAAGATCCTCTCTCGCTAATCTCCGCCACCGGCCCTTTATAATGC

GAGGGTCTGGAGGGCTGAGGACCCCGAGCTGTGCTGTGCTGCGGCCGCCACCGCGGG—CCCCGCGCTCTTTGGCTCCCTCTGCTCGAGAGAGGGCTTCTCAGAGGCTTGGCG

 GAGGGTCTGGAGGGCTGAGGACCCCGAGCTGTGCTGTGCTGCGGCCGCCACCGCGGGCCCCCGCGCTCCCTGGCTCCCTCTGCTCGAGAGAGGGCTTCTCAGAGGCTTGGCG

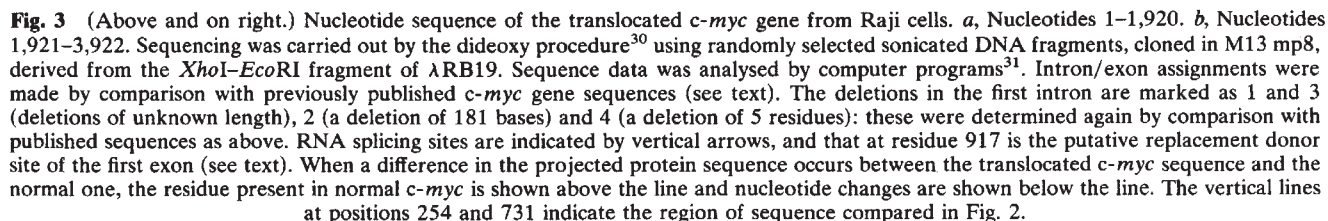
Xho
 GGAAAAAGAACGGAGGGAGGGATCGCTCTGAGTATAAAAGCCGATTTCGGGGCTTTATCTAACTCGCTGTAGTAATTTACCGAGAGGAGAGGGAGCGAGCGGGCGGCCCTAGGGT

 GGAAAAAGAACGGAGGGAGGGATCGCGCTGAGTATAAAAGCCGTTTCGGGGCTTTATCTAACTCGCTGTAGTAATTTACCGAGAGGAGAGGGAGCGAGCGGGCGGCCCTAGGGT

GGAGAGCGCCGGCGAGTAGAGTTGCACT-----TGGGAAGGGAGATCCGGAGCGAATCGGGGGCTTCGCTCTGCGCCAGCCCTCCCGCTGATCCCCAGGCGAGTGGTCCGCAAC

 GGAGAGCGCCGGCGAGCGAGCTGCGCTGCGGGCGCTCTGGGAAGGGAGATCCGGAGCGAATAGGGGGCTTCGCTCTGCGCCAGCCCTCCCGCTGATCCCCAGGCGAGTGGTCCGCAAC

Fig. 2 Comparison of DNA sequence from the 5' end of normal and translocated *c-myc* genes from Raji cells. The DNA sequence on the top line of each row represents the translocated *c-myc* gene sequence whilst the bottom line of each row represents the normal *c-myc* sequence. Stars between residues indicated identity and a short deletion in the translocated gene is shown by dashes. The position of an *XhoI* site (see text) is marked. DNA sequence was obtained by cloning *XhoI* plus *PstI* fragments in M13 mp8 and mp9²⁷ and sequencing by the dideoxy chain termination procedure^{28,29}.



A final point of interest from the Daudi cDNA sequence shown in Fig. 4 is that the 3' end extends for 172 nucleotides beyond the K562 *c-myc* cDNA sequence previously published¹¹. This demonstrates that RNA transcription can extend beyond the poly(A) addition site (located at position 1614 in Fig. 4) indicated by sequencing of *c-myc* genes. Genomic DNA sequence beyond the end of the cDNA sequence is not available

The data presented in Fig. 3 show that 16 coding differences, along with other silent base changes, occur within exon 2 of a translocated *c-myc* gene. These differences could, therefore, generate a substantially altered protein. Comparison with other *c-myc* sequences rule out the possibility that all of these differences could result from genetic polymorphism at this locus. The most reasonable explanation would seem to be that these changes have been introduced into the *c-myc* gene by somatic mutation as a result of the presence of this gene in an immunoglobulin locus. Apart from the insertion of a leucine codon (nucleotide 1,321 in Fig. 2) no stop codons or frameshifts exist in the protein coding segment of this 'mutated' gene. Some or

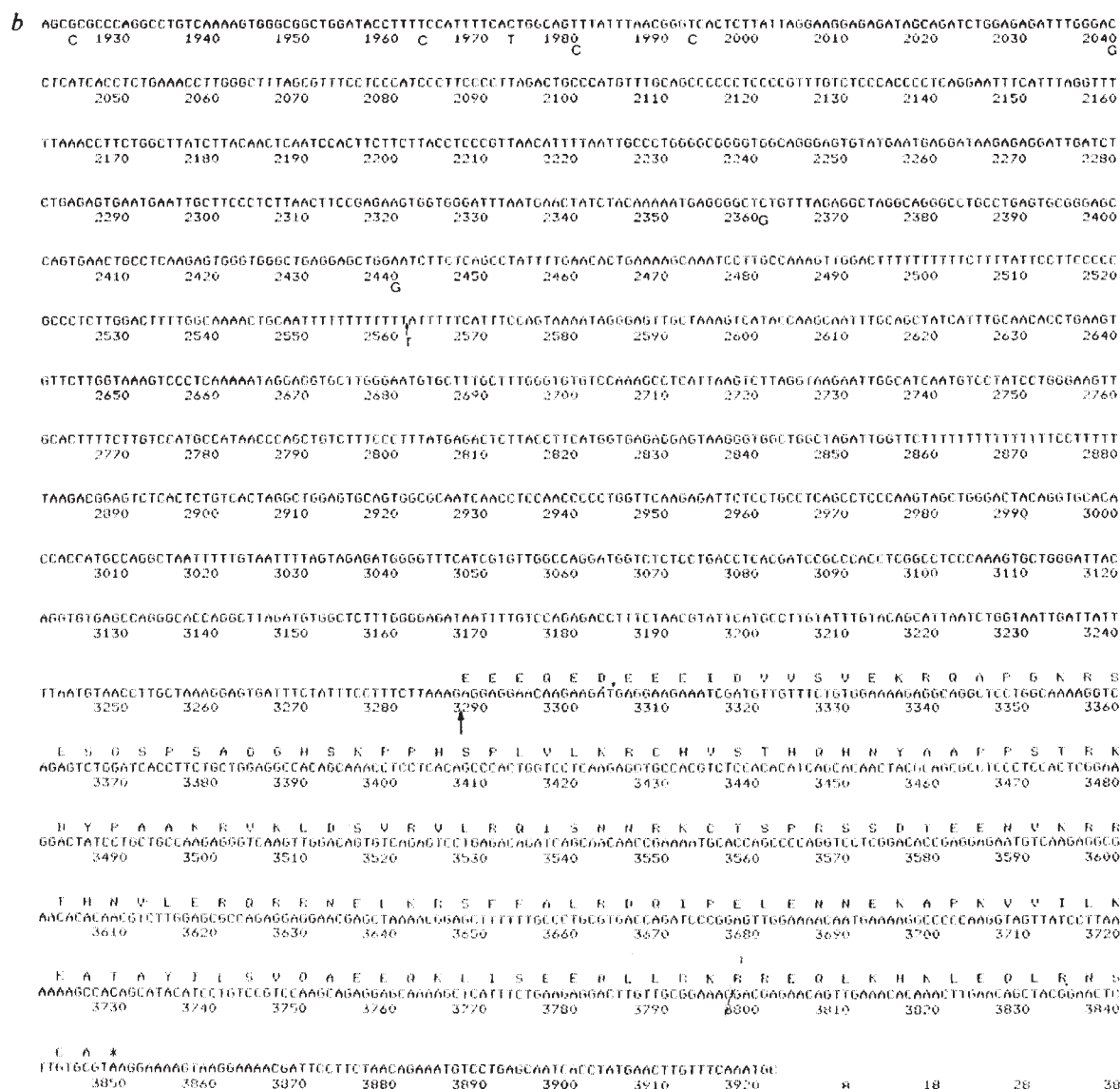


Fig. 3 (cont.)

all of the amino acid changes noted in the translocated *c-myc* gene may be important for Burkitt lymphoma oncogenesis. The large number of codon changes, however, make it difficult to assess the importance individual changes in the potential protein sequence. Table 1 lists the amino acid differences observed and compares them to chicken *c-myc*¹⁹ and *v-myc*^{20,21} sequences. When we compared the residues at which the Raji translocated *c-myc* differed from 'normal' *c-myc* to the corresponding residues from chicken *c-myc* and MC29 *v-myc* (Table 1) several of the differences in the translocated *c-myc* product correspond to amino acid residues not retained between normal human and chicken proteins (positions 7, 10, 88, 92, 203, 230, 240). More interesting are changes affecting amino acid residues that are maintained phylogenetically. Such changes can be divided into groups which are either conservative ones (positions 6, 39, 149) or non-conservative ones (positions 58, 114, 120, 245). It is worth noting that at position 58 there is a change from threonine to asparagine in the translocated gene: this corresponds to a position at which *v-myc* and chicken *c-myc* also differ. Residue 58 occurs in a 19 residue stretch that is perfectly conserved between normal human and chicken *c-myc* as well as the recently described Nb1-*myc* gene detected in neuroblastoma cells²². Furthermore, this region is also altered in the Raji translocated *c-myc* by the insertion of the leucine at position 56a. Changes in this whole region may, therefore, be important.

The codon changes detected in activated *c-ras* genes have so far been found to be limited to one of two positions²³⁻²⁶. This situation need not necessarily be paralleled in the *c-myc* changes, as amino acid replacements in or near an active site might be sufficient to cause disruption of normal function. It is possible, therefore, that regions of changes may be pertinent rather than any specific residue. Sequence data on other Burkitt lymphoma *c-myc* genes is needed before a definite conclusion can be drawn regarding the relevance of somatic changes in translocated *c-myc*. Recently it has been demonstrated that the chicken *c-myc* gene acquires somatic mutations as a result of integration of avian leukosis virus³² so such mutations may be a general phenomenon in these situations.

The important point is the possible significance of the amino acid replacements for oncogenesis in Burkitt lymphoma. It has been argued that elevated levels of *c-myc* mRNA are transcribed from the translocated allele²⁶ and that by implication this would be sufficient for the translocation of the *c-myc* gene to exert its effect on the leukaemic precursor cell. The observation of nucleotide changes in the translocated *c-myc* gene provides an alternative mechanism by which this *c-myc* gene could alter the cellular metabolism (whether or not in combination with elevated transcription levels). Apart from codon changes the significance of alterations to exon 1 is now under investigation.

Fig. 5 Schematic representation of changes existing in the translocated *c-myc* gene of Raji cells. The region represented extends from the methionine codon at the start of exon 2 until about the middle of the intron between exons 2 and 3. Vertical lines indicate either base changes (below) or codon changes (above). At the bottom is a scale showing the distances from the start of the switch region in the λ RB19 clone.

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LETTERS TO NATURE

Photon oscillations and cosmic background radiation

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The possible existence of a second species of photon which is uncoupled to known forms of matter is considered here. Explicit mass terms in the lagrangian can give rise to photon masses and to oscillations of photon identity, without sacrificing the ability of the gauge theory to be renormalized. Current upper limits on the photon mass are $\sim 6 \times 10^{-16} \text{ eV } c^{-2}$ (refs 1, 2). Photon oscillations corresponding to much smaller masses can significantly alter the spectral shape of the cosmic background radiation (CBR). Indeed, we show that the apparent discrepancy³ between theoretical and observed CBR spectra can be resolved in terms of photon oscillations, and a mass parameter of $5 \times 10^{-18} \text{ eV } c^{-2}$.

Our model of photon oscillations is a simple extension of the standard gauge theory of strong interactions wherein an additional gauged $U'(1)$ factor is adjoined to the usual spontaneously broken $SU(3) \times SU(2) \times U(1)$. Known particles transform trivially under $U'(1)$. At this point, the model involves two massless gauge fields, one of which is essentially undetectable. Introduction of explicit mass terms involving the $U(1) \times U'(1)$ gauge fields does not upset the renormalizability of the theory. For simplicity, we choose a degenerate mass matrix so that one photon eigenstate remains massless:

$$\begin{aligned} A(\text{standard photon}) &= a_1 \cos \phi + a_2 \sin \phi \\ B(\text{uncoupled photon}) &= a_2 \cos \phi - a_1 \sin \phi \end{aligned} \quad (1)$$

where the mass-eigenstate fields are given by $a_{1,2}$,

$$m(a_1) = 0, \quad m(a_2) = \mu (\text{tiny}) \quad (2)$$

Photons emitted by ordinary matter are initially type A, but their amplitude evolves as

$$a_1 \cos \phi + a_2 \sin \phi \exp(-i\mu^2 c^4 t / 2\hbar E) \quad (3)$$

so that a B component develops with time. The probability that an ordinary photon remains an ordinary photon thus oscillates with time according to

$$p(t) = 1 - \sin^2(2\phi) \sin^2(\mu^2 c^3 \lambda t / 8\pi \hbar^2) \quad (4)$$

where λ is the photon wavelength. These oscillations of photon identity are entirely analogous to often-discussed but never-seen neutrino oscillations.

Certain upper limits on the photon mass also constrain the possibility of photon oscillations. From the analysis of refs 1, 2, we conclude that

$$\mu \sin^2 \phi < 8 \times 10^{-49} g = 6 \times 10^{-16} \text{ eV } c^{-2} \quad (5)$$

Our principal observation is that a smaller value of μ can have a significant impact on the spectral shape of the CBR.

Measurements of the CBR^{3,4} are in crude agreement with a 3 K black-body distribution. In our picture, there are two components (A and B) to the CBR which are subject to the oscillations described by equation (4). Not having specified the spectra of particles bearing B-charge, we are free to vary both the time of B-photon decoupling and the amount by which the B-photon temperature shifts with respect to the A-photon temperature due to entropy release as the cosmological temperature falls through various mass thresholds. Thus the temperatures of the two components of the CBR are relatively unconstrained. The distribution of interacting photons today is given by

$$\begin{aligned} P(\lambda, T_A) + \{P(\lambda, T_B) - P(\lambda, T_A)\} \\ \times \sin^2(2\phi) \sin^2(3\mu^2 c^3 \lambda \tau / 40\pi \hbar^2) \end{aligned} \quad (6)$$

where $P(\lambda, T) = 4\pi \hbar c^2 \lambda^{-3} / (\exp(2\pi \hbar c / \lambda k T) - 1)$ is a Planck distribution corresponding to temperature T , and $T_{A,B}$ are the temperatures of the two photon species. To take into account the redshift in a mass-dominated universe, we have substituted $i\mu^2 c^4 \int \tau dt / 2\hbar E(t)$, $E(t) \sim t^{-2/3}$, in the argument of the exponential of equation (3). Here $\tau \approx 1.3 \times 10^{10} \text{ yr}$ is the age of the Universe, compared with which all other time scales may be neglected. Despite the non-vanishing mass μ , the Planck distributions of equation (6) remain those appropriate for only two photon polarization states since the extra longitudinal mode is effectively decoupled throughout the frequency range in which measurements are taken.

Our result, equation(6), can give a better description of the observed CBR spectrum for a variety of values of T_A , T_B and $\sin^2(2\phi)$, providing that we take

$$\mu \approx 5 \times 10^{-18} \text{ eV } c^{-2} \quad (7)$$

One choice of values, suggested by the currently available data, is displayed in Fig. 1 together with the conventional fit³, $P(\lambda, 2.96 \text{ K})$. It is, of course, not surprising that the CBR can be better fitted with four parameters than with one, and we cannot claim that photon oscillations have been seen. However, if photon oscillations are the cause of the spectrum anomaly, they can have spectacular and observable effects in the microwave domain if measurements can be done with small enough bandwidth. Figure 2 shows the shape of the CBR in the region $\lambda < 2 \text{ cm}$ for an idealized zero bandwidth experiment, using the same parameters as in our fit of Fig. 1, and showing the curious sinusoidal variation in flux as a function of wavelength. Equally good fits to the observed CBR in Fig. 1 can be obtained with

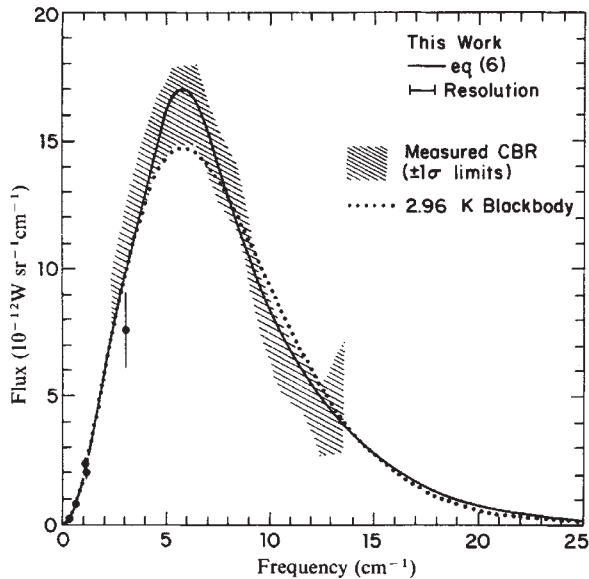


Fig. 1 Predicted spectrum of the cosmic background radiation (solid line) based on equation (6) with $T_A = 3.17$ K, $T_B = 2.0$ K, $\sin^2 2\phi = 0.4$, $\mu = 5 \times 10^{-18}$ eV c^{-2} , and bandwidth averaged as in ref. 3. The $\pm 1\sigma$ flux limits (shaded area) of ref. 3 and other data points are taken from ref. 4. The spectrum of an ordinary 2.96 K blackbody (dotted line), which best fits the integrated flux of ref. 3, is also shown for comparison.

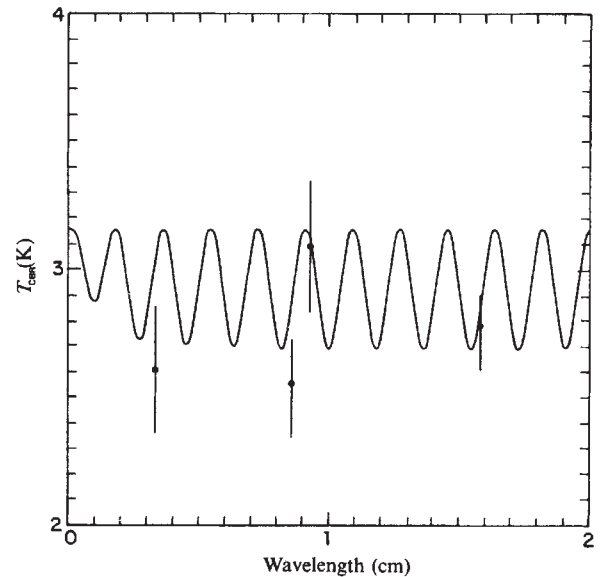


Fig. 2 Predicted microwave cosmic background flux plotted as an effective temperature determined by inverting the Planck distribution at each wavelength. The spectrum is calculated from equation (6) using the same parameters as used in Fig. 1 but assuming zero bandwidth (our value of the mass μ corresponds to $3\mu^2 c^3 \tau / 40\pi \hbar^2 = 5.5 \pi \text{ cm}^{-1}$ in equation (6)). Experimental values are again from ref. 4.

different values of T_A , T_B and $\sin^2 2\phi$, which give oscillations in Fig. 2 with smaller amplitude but with equal period.

We have not taken into account the possible existence of an extragalactic free electron plasma of density n . A-type photons develop an effective mass proportional to the plasma frequency (m is the electron mass)

$$\mu_A = [4\pi n e^2 \hbar^2 / m c^4]^{1/2} \quad (8)$$

which, if larger than μ , extinguishes the photon oscillations. Our value $\mu_A \leq 5 \times 10^{-18}$ eV c^{-2} hence requires that

$$n \leq 10^{-14} \text{ cm}^{-3} \quad (9)$$

While $n \sim 10^{-3} \text{ cm}^{-3}$ within our Galaxy, there are only upper limits to extragalactic densities. The Gunn-Peterson test⁵, for example, limits the extragalactic density of neutral hydrogen to $\leq 2 \times 10^{-12} \text{ cm}^{-3}$. Thus equation (9) is compatible with all observational data. We propose no theory of galaxy formation which predicts such a small extragalactic electron density, nor are we aware of one which definitively excludes it.

If a CBR photon passes through one or more galaxies on its way to Earth, oscillations will be interrupted and suppressed. Nonetheless, it is not necessary that the line-of-sight be evacuated all the way back to the cosmic photosphere. Photon oscillations begin only when the electron density falls to the value given by equation (9), a time certainly after galaxy formation. Thus, the lower limit to the phase integral $\int dt/E(t)$ should be taken to be a small fraction of τ , and can always be compensated with a small change in μ in our final result. Because the redshift of the energy denominator weights later times more heavily, even the choice of $\tau/8$ as a lower limit turns out to reduce the phase integral by $< 5\%$. This initial time corresponds to a redshift of $z \sim 3$, since which time (it is estimated⁶) the majority of photons reaching Earth have passed through no galaxies. Those photons that have encountered galaxies are likely to have done so rather early and have in any event spent a negligible total time in galactic transit. The incorporation of these effects leads to virtually no change in Fig. 1, and a small decrease in the already uncertain magnitude of the oscillations of Fig. 2.

The existence of photon oscillations or of a small photon mass could indicate that there is no unified theory of all the forces. In a unified theory, all the gauge interactions are included in a

simple group. Explicit gauge boson mass terms spoil renormalizability, so these masses must be introduced through the Higgs mechanism. Photon masses are then of the order of gQv where g is the universal gauge coupling constant, Q is the dimensionless charge of the Higgs field (of order unity), and v is its vacuum expectation value. The mass of the surviving Higgs boson is of the order of $\lambda^{1/2} v$ where λ is the Higgs self-coupling.

In order that this theory behave as a theory with a massive photon, the Higgs boson must be very heavy and thereby removed from the effective ordinary energy theory. Otherwise, the Higgs particle combines with the longitudinal component of the massive photon to act like a light electrically charged scalar meson. Such a large Higgs mass can only be achieved by taking v to be very large compared with ordinary particle masses ($> 1 \text{ GeV } c^{-2}$). A photon mass roughly 30 orders of magnitude smaller than this, then, requires Q to be extremely small. Only in an abelian theory (such as the $U(1) \times U'(1)$ portion of our non-unified model), however, can the charge Q be so chosen. In a simple gauge group, Q is integrally quantized and consequently the Higgs boson mass cannot be much larger than the photon mass terms.

Thus we see that any unified theory incorporating small photon masses must also contain an experimentally excluded light-charged meson. Observation of photon oscillations, then, would require that the gauge group of the world contain one or more abelian factors. This is one of the rare cases in which low-energy information can constrain the structure of the theory at arbitrarily high energies.

The phenomenology of our model is similar to that considered by Okun⁷. A similar model has also been considered by A. Zee and S.L.G. (unpublished).

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Low-frequency jovian emission and solar wind magnetic sector structure

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The Earth, Jupiter and Saturn emit non-thermal low-frequency radiations with similar characteristics. For the Earth and Saturn, the radio emissions are known to fluctuate with a time scale of several days, correlated with variations of the solar wind or related phenomena at the planet¹⁻⁴. Several studies of the jovian radiation at decametre wavelengths, from ground-based observations, suggest that the non-Io-controlled emission fluctuates in response to the sector structure of the interplanetary magnetic field⁵⁻⁹. We study here the long-term fluctuations of the jovian emission at hectometre and kilometre wavelengths. We use observations from the Planetary Radio Astronomy (PRA) experiment aboard the two Voyager spacecraft. We show that these emissions are strongly affected by the magnetic sector structure at Jupiter. This leads us to discuss the position of the sources of emission in the jovian magnetosphere.

The ground-based systematic monitoring of Jupiter at decametre wavelengths (8–10 to 40 MHz) has shown two dominant fluctuations in the emission, one at the rotation period of the planet and the other at the orbital period of satellite Io. Ground-based data are also modulated by the rotational and orbital periods of the Earth, which limit the observation times, and make the data sets very discontinuous. Therefore, a systematic search for periodicities by power spectrum analysis showed no significant feature at the solar rotation period seen from Jupiter, which is about 25.5 days (ref. 10). However, using superposed epoch analysis⁶⁻⁹ and cross-correlation techniques⁵, jovian decametre activity was compared with the dynamic pressure of the solar wind and to various indexes of geomagnetic activity at the Earth, extrapolated to Jupiter. It was concluded that non-Io-controlled emissions fluctuate in association with the interplanetary sector structure.

The PRA experiment aboard the Voyager spacecraft observed Jupiter's emission for several years, with nearly continuous time coverage in the lower band of observation, from 1.2 kHz to 1.3 MHz (emissions in the high-band of the receiver were blurred by spacecraft interference except close to encounter). Moreover, at these low frequencies, no effect of Io has been found, so the low-band data are clear of most modulations which interfere with the study of periodicities from the ground-based data. We now consider the period from 15 December 1978 to 5 July 1979. Voyager 1 (V1) and Voyager 2 (V2) encountered Jupiter on 5 March and 9 July 1979 respectively.

To study the temporal variations of the emission with a time scale of several days, we have plotted the dynamic spectrum of the emission using a condensed time scale. Figure 1 shows observations performed for a 22-day period, after V1 encounter but before that of V2. During these 22 days the two spacecraft viewed the planet from very different angles. In Fig. 1, the hectometre emission of Jupiter, which is thought to be the extension of the decametre emission emitted at higher altitudes, is seen as vertical dark streaks extending from the highest frequency limit of the receiver. The kilometre emissions are mostly between 0.1 and 0.3 MHz and occur between episodes of hectometre emission. The dominant short-term modulation which appears in Fig. 1 is due to the planetary rotation, which produces vertical features in the spectra with a period of 9 h 55.5 min. But large fluctuations of the intensity of the emission are also seen with a time scale of several days. They affect the

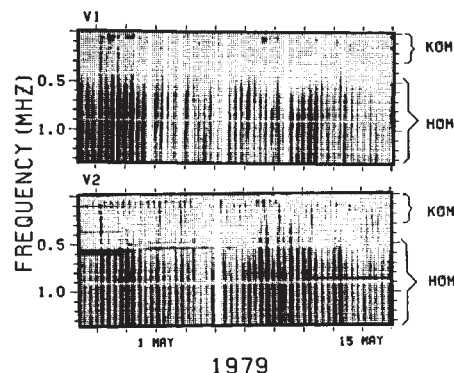


Fig. 1 Compacted dynamic spectrum of jovian emission observed by Voyagers 1 and 2 in the low band of the Planetary Radio Astronomy receiver. The two spacecraft were at similar distances from the planet, with Voyager 1 above the post-midnight quadrant at about 0400 LT and Voyager 2 in the pre-noon sector at approximately 0930 LT. The emission appears black on a grey background. White areas represent data gaps. Horizontal features are spacecraft interference. Intense solar type III are seen as vertical lines covering most of the frequency range, for example, 26 April 1979 close to 1200 LT. Periods of intense emissions are seen from about 27 April to 30 April and 8 to 12 May, while low intensities occur from about 3 to 8 May and from 14 to 17 May. KOM, kilometre; HOM hectometre wavelengths.

whole observed frequency range, and are seen simultaneously from both spacecraft. This type of long time scale fluctuations has already been pointed out in the case of broad-band kilometre radiation¹¹.

The long-term fluctuations of the intensity observed by the two spacecraft at hectometre wavelengths are shown on Fig. 2a; here the whole 6.7-month time-interval of the study is included. There is a good correlation between the two curves, even though the viewing geometries differ considerably from the time of V1 encounter until that of V2.

The case of the kilometre emission is more complex; strong solar type III bursts, negligible at higher frequencies, can be more powerful than the jovian emission, except close to encounters; as shown on Fig. 2b, the fluctuations observed are best correlated with those seen at hectometre wavelengths close to the encounter.

Two components of the emission are included in Fig. 2b: the narrow-band component; confined at low frequencies, and the broad-band one, expanding towards higher frequencies¹². We have verified that the intensity fluctuations at frequencies where only broad-band kilometre emission occurs (with weak intensity) are similar to those found on Fig. 2b. This implies that the broad-band kilometre emission exhibits fluctuations correlated to those at hectometre wavelengths. It is not yet clear whether a similar correlation exists for narrow-band kilometre emissions.

To look for the origin of these fluctuations, we have performed a power spectrum analysis of the data. The result for hectometre emission is displayed on Fig. 3. Several significant peaks are observed: close to 29 days (+2.5 days–2.0 days), 14.5 days, 10.5 days, 7.2 days (the last significant for V1 only). Some of the peaks have different relative intensities for the two spacecraft.

For most of the time studied, the solar magnetic field polarity pattern showed a well defined structure. Beginning on 6 February 1979, after a disturbed period, there were two unequal sectors whose durations evolved slowly in the ranges 14–16.5 and 9–11 days respectively. The lengths of the two sectors corresponded well to the peaks at 14.5 and 10.5 days found in the power spectra. The ~29-days period is longer than the solar rotation period as observed from Jupiter (25.5 days); this may be due to the longer period of active solar phenomena close to a solar maximum, as in 1979. Taking into account error bars, it is very close to the ~26.5-days period found when studying

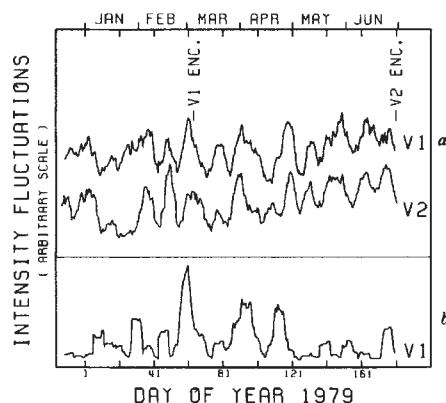


Fig. 2 Intensity variations observed: *a*, by the two spacecraft at hectometre wavelengths; *b*, by Voyager 1 at kilometre wavelengths. Systematic intensity variations due to changes in the distance R from the spacecraft to Jupiter have been corrected according to a $1/R^2$ law. The modulation due to Jupiter's rotation has been eliminated by performing an average over each rotation, and fluctuations shorter than 6 days have been smoothed by a running mean. The hectometre curves are obtained by averaging 13 'clean' intensity channels chosen between 0.788 and 1.3 MHz, the kilometre curves by averaging three channels between 78 and 116 kHz.

the fluctuations of the distant magnetotail of the planet in 1980¹³, and like this study, we conclude that this period is of solar origin. The peak at 7.2 days is likely to be an harmonic of both peaks at 29 and 14.5 days, which makes it stronger than a possible harmonic of the 10.5-day peak.

From the evidence presented above, we conclude that the sector structure of the interplanetary magnetic field has an important role in controlling the long-term intensity fluctuations observed at hectometre wavelengths, and also the correlated variations of the broad-band emissions at kilometre wavelengths. It was already known that the solar wind influences the jovian radio emissions at decametre wavelengths. Moreover, modulation of the radiation was observed simultaneously by Voyagers 1 and 2 with their different viewing geometries. This implies that global changes of the radiating region due to solar wind variations are involved.

It is generally thought that the magnetosphere of Jupiter is dominated by rotational dynamics¹⁴, contrary to the case of the Earth where the effect of the solar wind is dominant. But it has been shown¹³ that the structure of the jovian magnetospheric tail exhibits variations with a period of ~ 26.5 days, and is therefore apparently controlled by the solar wind structure. The solar wind might also have a significant influence on other parts of the magnetosphere, as the magnetically connected polar caps which have not been explored by spacecraft¹⁴. As we have established that the jovian low-frequency radio emission is also modulated by the solar wind structure, a relationship certainly exists between the source(s) of the emission and the regions where the solar wind has an important role. No direct observational information is known about the position of these sources. They are usually thought to be on field lines intersecting the Io-torus where electron acceleration can occur. But the torus is imbedded deep in the inner jovian magnetosphere, and there is no information on the possibility of significant variations in the Io-torus properties with a time scale of several days, nor do we know how solar wind might induce such variations. This leads us to question the usual hypothesis that the non-Io-controlled sources of emission are located on field lines connected with the Io torus. Furthermore, the acceleration of electron beams can occur in the tail or at polar caps—which as mentioned above are known or supposed to be controlled by the solar wind structure—as plausibly as in the Io torus. A complete comparison of the temporal and spectral variations of the Io and non-Io controlled emissions of Jupiter, from the different viewpoints obtained during the Voyager missions, might allow us to answer this question. Anyway, the role of the

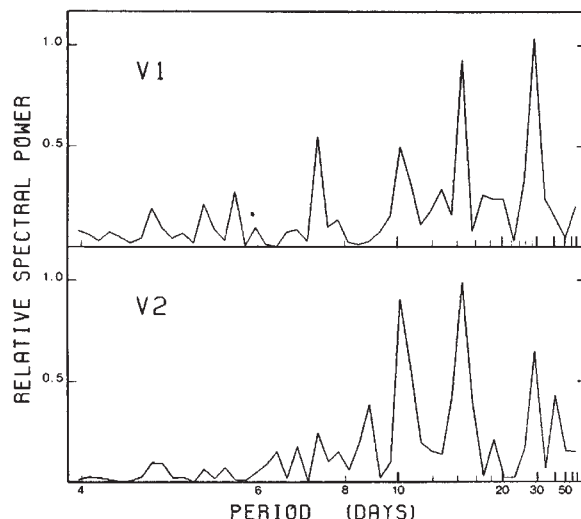


Fig. 3 Power spectrum of the intensity variations observed by Voyagers 1 and 2, calculated after correcting for variations due to changes in the Jupiter-spacecraft distance and smoothing the fluctuations shorter than 2 days.

solar wind in the jovian magnetosphere seems to be more important than previously thought, although it remains secondary to the role of rotation.

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Combustion-generated carbon particles in the Arctic atmosphere

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Recent studies in the Alaskan Arctic^{1–3} show the presence of substantial concentrations of carbon- and sulphur-containing particles that seem to be characteristic of the Arctic region as a whole^{4–6}. These particles are effective scatterers and absorbers of visible radiation^{3,7} and appear to be responsible for the phenomenon of Arctic haze first reported by Mitchell⁸. On the basis of trace element analysis, it has been suggested that these particles originate from anthropogenic sources at mid-latitudes^{1,4}. Direct substantiation of combustion-generated particles in the Arctic atmosphere has been provided by the identification of large concentrations of graphitic carbon particles at the GMCC-NOAA (National Oceanic and Atmospheric Administration) observatory near Barrow, Alaska, using Raman spectroscopy³. We report here on an extension of our

studies of carbon particles in the Alaskan Arctic to the Canadian Arctic and the Norwegian Arctic. These studies, using the Raman scattering technique, identify substantial concentrations of graphitic carbon particles at ground-level stations throughout the western Arctic.

Graphitic particles can only be produced by combustion processes. If one excludes natural burning processes that are not expected to be a significant source during winter and spring when Arctic haze is at a maximum, then one can attribute these particles directly to anthropogenic combustion sources. These particles, which have large absorption cross-sections ($\sim 10 \text{ m}^2 \text{ g}^{-1}$) in the solar spectral region, could lead to significant heating effects over the high surface albedo polar ice-cap⁹⁻¹¹. The magnitude of these effects largely depends on the vertical and horizontal distributions of the graphitic particles as well as their concentrations as a function of time of year^{10,11}.

The Raman spectroscopy apparatus uses a coherent radiation argon ion laser producing 1 W of power at 514 nm. The laser beam is focused by a 75-mm focal length cylindrical lens to a spot $0.06 \times 2 \text{ mm}$ on the sample surface through a small mirror, and the backscattered radiation is collected and imaged by an $f/1$ lens onto the slit of a 1-m Jarrell Ashe double monochromator equipped with two $1,180\text{-grooves mm}^{-1}$ gratings blazed at $5,000 \text{ \AA}$. The output of the spectrometer is detected by an FW130 photomultiplier cooled to -20°C and used in a photon-counting mode. The pulses, after appropriate shaping, are counted and displayed on a multichannel analyser. A computer-controlled grating drive (RKB, Inc.) allows a given spectral region to be scanned many times and added to the memory of the multichannel analyser, greatly improving the signal-to-noise ratio. To minimize heating effects, the highly absorbing samples used in these experiments are rotated at $1,800 \text{ r.p.m.}$ by a motor, which increases the area illuminated by the laser beam by a large factor with almost no loss in signal level. The focal spot of the laser is located $\sim 5 \text{ mm}$ below the axis of rotation so that the effective illuminated area is an annulus of 5-mm radius and 2-mm width, resulting in a power density of $\sim 1 \text{ W cm}^{-2}$. With this low-power density, no blackening of the filter deposit due to pyrolysis was observed.

The Raman spectra are obtained directly from aerosol particles collected on various filter media without any pretreatment. These spectra are observed on top of a large fluorescent background, which is due both to the filter media and the highly fluorescent material in the sample. Irradiation of the sample with the argon laser for 24 h reduced this background by about an order of magnitude. The intensity of the Raman spectra were typically about 1% of the fluorescent background. Aerosol samples were obtained from six Arctic sites: Barrow in the Alaskan Arctic; Mould Bay, Igloolik, and Alert in the Canadian Arctic; and Bear Island and Spitzbergen in the Norwegian Arctic. The samples from the Alaskan Arctic were collected on prefired quartz-fibre filters (Pallflex 2500 QAO) with a sampler installed at the NOAA-GMCC observatory; the samples from the Canadian and Norwegian Arctic were collected on Whatman 41 filters with samplers installed respectively by the Canadian Atmospheric Environment Service and the Norwegian Institute for Air Research.

Raman scattering and IR absorption spectroscopy are complementary techniques that measure the vibrational spectra of gases, liquids and solids. Often vibrational modes that are IR inactive are Raman active and vice versa. Graphitic structures in which carbon atoms occupy lattice sites in a two-dimensional hexagonal honeycomb network have intense Raman modes but very weak IR vibrational spectra. These Raman modes, which were first observed by Tuinstra and Koenig¹², enable the identification of graphitic structures even in the presence of a complex mixture of substances. Solvent extraction, heat treatment, optical absorption and morphology studies¹³ can provide indirect evidence for a graphitic component; but Raman spectroscopy seems to be the only available method for making unambiguous identifications on a molecular level.

The Raman scattering technique has been applied to identify

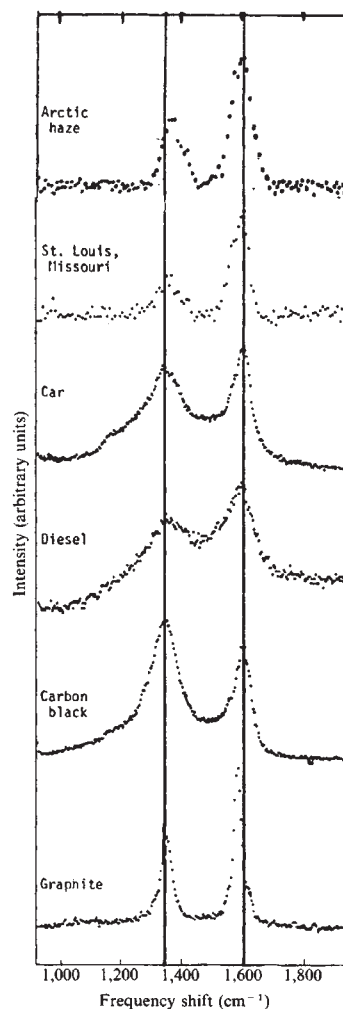


Fig. 1 Raman spectra of particles collected in the Alaskan Arctic compared with spectra of urban particles, various source emissions, carbon black, and polycrystalline graphite.

substantial concentrations of graphitic particles in combustion effluents, urban air^{14,15}, and the Alaskan Arctic³. These results are shown in Fig. 1, where the spectrum of a sample collected in December 1979 at the NOAA-GMCC observatory in the Alaskan Arctic is compared with spectra of urban particulates, various source emissions, carbon black, and polycrystalline graphite. All spectra show the presence of two intense Raman modes that have been attributed to phonons propagating within graphitic planes¹². The Raman spectrum of a single crystal of graphite consists of a single band near $1,575 \text{ cm}^{-1}$, which has been identified as an E_{2g} vibration of the hexagonal layers¹². Polycrystalline graphite shows another band at $1,355 \text{ cm}^{-1}$, which has been attributed to the breakdown of selection rules due to a loss in extended crystal symmetry¹². A small shift in the frequency of the $1,575 \text{ cm}^{-1}$ Raman mode has also been observed, which apparently depends on the degree of order in the aromatic structures¹⁶. Such a shift is evident in comparing the spectra of polycrystalline graphite with the other spectra presented in Fig. 1. It is also evident from Fig. 1 that the frequency of the vibrational modes in carbon black and those in particles collected from various sources, urban air and the Alaskan Arctic, are coincident to $\pm 10 \text{ cm}^{-1}$, which is the estimated experimental error. This close correspondence has been used to show the presence of graphitic particles in urban air¹⁴ and in the Alaskan Arctic³. Figure 2 extends these measurements to samples collected from three sites in the Canadian Arctic (Mould Bay, Igloolik, Alert) and two sites in the Norwegian Arctic (Spitzbergen, Bear Island). All samples were collected at similar times of the year but due to sample availability, some samples are from 1980 and others from 1981. It is clear from

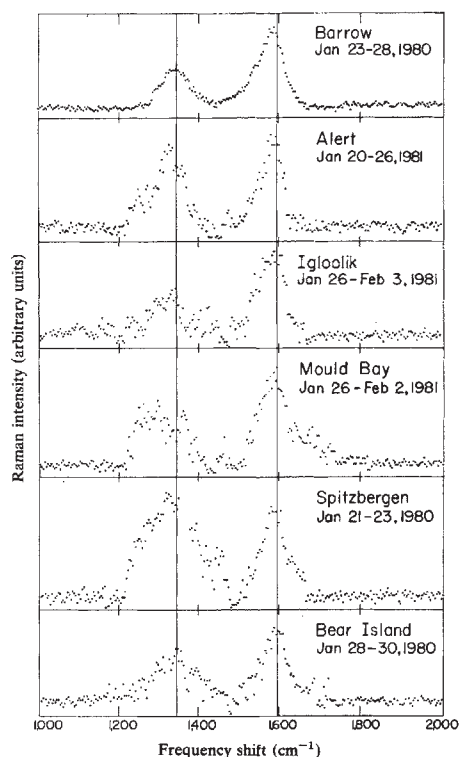


Fig. 2 Raman spectra of particles collected in Alaskan Arctic (Barrow) compared with samples collected in the Canadian Arctic (Alert, Igloolik, Mould Bay) and the Norwegian Arctic (Spitzbergen, Bear Island).

the spectra that all sites show the presence of significant concentrations of graphitic particles. There are some differences in the relative intensities and line shapes of the two Raman modes, but these could be due to systematic errors in fluorescence subtraction for the $1,350\text{ cm}^{-1}$ mode, which is located on a highly sloping background. If the loadings on the filters are sufficiently low to avoid saturation effects, as in the samples used in these experiments, and one assumes fixed optical constants (Raman cross-sections, absorption cross-sections), then the integrated intensity of the $1,600\text{ cm}^{-1}$ Raman mode can be used as a measure of the relative concentrations of graphitic particles at these sites. These analyses indicate that the concentrations at all these sites are quite comparable with the largest and smallest within about a factor of 3 of each other. The relative ordering of these concentrations for Spitzbergen, Bear Island, Barrow, Mould Bay, Alert and Igloolik is 2.1/1.7/1.0/0.8/0.8/0.7. Note that this analysis is for a particular time interval, and the relative contributions could vary considerably from one time period to the next.

These results show that the large concentrations of graphitic particles found at the Barrow, Alaska, site are not a local phenomenon but are characteristic of ground-level stations across the western Arctic. If these highly absorbing particles occur at substantial concentrations throughout the Arctic troposphere, then they may have a significant impact on the Arctic radiation balance and climate.

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Catalytically grown carbon filaments from a smelter aerosol

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Aerosol particles consisting of complex intergrowths of carbon, copper and zinc occur in the plume downwind of a copper smelter. Using scanning and transmission electron microscopy we analyse their chemical and structural properties, and demonstrate how such an analysis can be used to fingerprint the source of atmospheric pollutants. The chemical and structural characteristics provide unique and highly specific information about their origin and catalytic growth mechanism.

Fine-grained anthropogenic particles constitute a substantial fraction of the global aerosol, especially near densely populated regions. Their small dimensions make detailed study difficult, and thus the number of chemical studies of individual particles has been limited. Most emphasis has been on bulk chemical analyses, but they provide only indirect and therefore limited information about the properties of the individual particles within a sample. However, many important chemical and physical effects of aerosols, including health effects, are determined at the single particle level and so it is desirable to learn as much as possible from individual particles¹⁻⁴.

Using both analytical scanning and transmission electron microscopy (SEM and TEM), we have developed procedures that allow characterization of individual aerosol particles^{5,6}. These methods have enabled the description of the chemical and physical evolution of selected particle types, providing an unambiguous means of fingerprinting specific sources of particulate pollution⁷. Furthermore, it has been possible to characterize surface microdeposits on individual, respirable particles⁸, and information about the surface chemistry of such particles can be used to assess their potential toxicity^{9,10}.

We have used a range of electron-beam methods to characterize particles emitted from an Arizona copper smelter. Their unusual morphologies and fibrous shapes make them of special interest; they also provide a good illustration of the unique types of information that can be obtained from individual-particle analyses.

The particles of interest consist of individual and clumps of fibres. All of the specimens we studied were airborne, but clearly the larger clusters settled out more rapidly than the single fibres, and an example is shown in Fig. 1. It consists of a large number of filaments that radiate outwards from a common base. Filament dimensions span the entire respirable size range, with diameters between 10 and 2,000 nm and lengths between 10^2 nm and 1 mm. Aspect ratios >20 are typical.

Structural features of the filaments were investigated by TEM. Instruments used for this study included: a JEOL JSM-35 scanning electron microscope, equipped with an X-ray crystal spectrometer and a Tracor Northern TN-2000 X-ray energy-dispersive spectrometer; a Phillips 400 T scanning transmission

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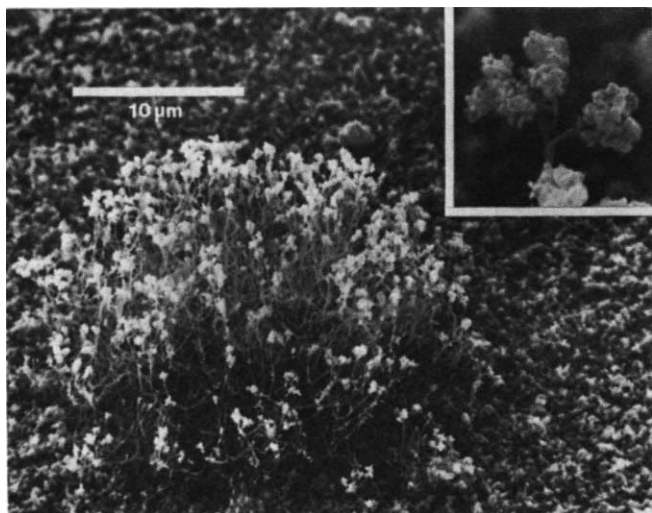


Fig. 1 Scanning electron micrograph of a filamentous carbon cluster (mounted on a Nuclepore substrate). This particle consists of several hundred carbon filaments that radiate outwards from a copper/zinc alloy base. The end of each filament (inset) contains a small 'flower' that consists of a copper/zinc grain embedded within the carbon.

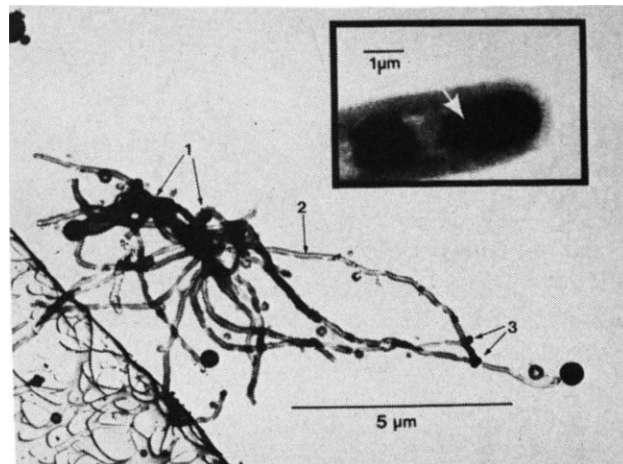


Fig. 2 Transmission electron micrograph of a filamentous carbon cluster. Arrow 1 indicates the copper/zinc alloy base of the cluster. Arrow 2 points to the hollow core structure of individual filaments. Arrow 3 shows copper/zinc alloy particles embedded at tips of filaments. The mesh structure, lower left, is part of a holey-carbon support film. Inset (upper right) is a high-magnification image of the tip of an individual carbon filament, showing a copper/zinc particle embedded within carbon.

electron microscope, equipped with a Tracor Northern TN-2000 X-ray energy-dispersive spectrometer and a Gatan Model 607 electron energy-loss spectrometer; and a JEOL JEM 100 B high-resolution transmission electron microscope. Images of individual filaments indicate a sheath surrounding a hollow core (Fig. 2). High-resolution images of the sheaths reveal 0.34 nm lattice fringes. These spacings, together with analyses by electron energy-loss spectroscopy, indicate that the filaments are composed of graphitic carbon. TEM images of the tips of individual filaments reveal pear-shaped masses embedded within the carbon (Fig. 2, inset).

Chemical variations within the particles were investigated by analytical SEM. X-ray energy-dispersive spectra, collected from various regions of the particles, indicate high concentrations of copper and zinc within the pear-shaped masses, as well as in the bases from which the filaments radiate (Fig. 2). Selected-area electron diffraction patterns indicate that both consist of a copper/zinc alloy. Back-scattered electron images of the clusters confirm that the tips of individual filaments as well as the bases of the clusters contain regions of high electron density material (copper/zinc alloy), whereas the filaments themselves consist of low electron-density material (carbon).

The analytical data, combined with published results about the growth of carbon filaments, provide considerable insight into these complex airborne particles. Although such particles have not previously been reported as constituents of an aerosol, the growth of carbon filaments has been extensively investigated in laboratory conditions¹¹⁻¹⁴. Baker and Harris¹¹ indicated that carbon filaments are produced by catalytic decomposition of carbon-containing gases between 327 and 1,027 °C. The mechanism of filament growth in these conditions is illustrated in Fig. 3. Initially, a carbon-containing gas (usually carbon monoxide or a hydrocarbon) decomposes on the exposed face of a metal catalyst particle. The carbon dissolves in the metal and is precipitated on the rear face to form the body of the growing filament. In this way, the filament grows upwards from the substrate, carrying with it the catalyst particle at its tip. Growth continues as long as the rate of diffusion of carbon away from the exposed face of the catalyst particle exceeds the rate of accumulation. The product of this reaction is an elongated single crystal of carbon containing a small metal catalyst particle embedded at its tip (see Figs 2, 3).

The particles were recovered from the atmosphere downwind of a copper smelter. There are several high-temperature pro-

cesses employed during copper smelting that utilize carbon-containing fossil fuels in the presence of catalytically-active materials. (For a description of the copper smelting process see refs 15, 16.) However, only one of those smelting processes, fire refining, provides the necessary combination of materials and environment to account for the observed properties of the particles under investigation.

During fire refining, 'blister copper' (a melt at ~1,150 °C that contains roughly 97.5 wt % copper) is purified during a two-stage redox procedure. During an initial oxidation step, compressed air is circulated through the melt to oxidize sulphide impurities to sulphur dioxide gas. Oxides in the melt are removed when the compressed air is replaced by a mixture of hydrogen and propane gas.

Fire refining provides an ideal environment for the growth of carbon filaments, and so large quantities of 'soot' and filamentous carbon are emitted during this process. The enrichment of zinc on the surface of the melt⁷ provides important information regarding the origin of the particles under investigation, since the compositions of the alloy at the bases of the filamentous clusters and at the tips of individual filaments (Fig. 2) correspond to the melt composition at the surface of the blister copper. The carbon filaments presumably formed during the reduction event, when propane circulated across the copper-zinc surface of the blister copper. Furthermore, the presence of hydrogen gas within the refining vessel would have a catalytic effect on the rate of filament growth¹¹. Thus, the observed characteristics of the aerosol particles under investigation are consistent with their origin within the fire refinery of the smelter.

Although we describe here the characteristics of only one particle type, a variety of particles within polluted atmospheres carry detailed information about their origins and subsequent evolution^{7,8}. For the example we have chosen, electron-beam microanalyses of the filaments indicate: (1) their gaseous origin, (2) the catalytic reaction mechanism responsible for filament growth, (3) the alloy that catalysed the reaction, (4) the exact stage within the smelting process when the particles were emitted. Such detailed work is clearly not appropriate for all types of aerosol particles. However, there are numerous instances where specific sources emit unusual types of particles. In such cases, as in the instance of the fibres we describe here, the detailed information available from the electron-beam study of individual fibres can provide a useful and even unique insight into the dynamics of airborne particle formation as well as a

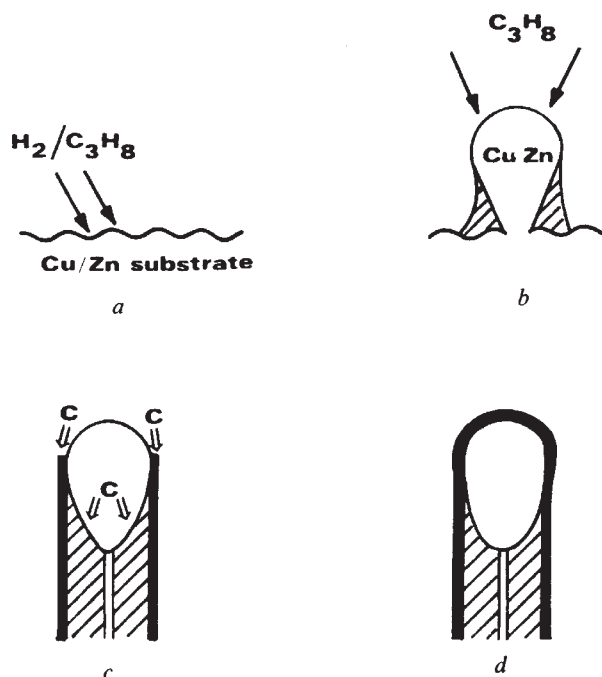


Fig. 3 Stages in the growth of filaments (modified after ref. 12). *a*, Mixture of hydrogen and propane passes over agitated surface of 'blister copper'; *b*, propane decomposes on the exposed surface of catalytically active alloy; *c*, carbon dissolves in alloy droplet, diffuses through droplet, and is precipitated on rear face to form body of growing filament; *d*, growth of filament terminates when catalytically active face is no longer exposed.

highly specific means of 'fingerprinting' sources of particulate pollution.

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The macromolecular states of solvent-swollen and dry coal

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Improved understanding of the physical state of coal is needed to better characterize, modify and utilize this abundant source of energy. In spite of extensive research in this area for well over half a century, the level of understanding has been poor. Here, new substantive evidence clarifying the macromolecular states of coal is presented. The approach involved studying changes in the optical anisotropy of raw and solvent-swollen thin section samples of a bituminous coal. It was found that the natural optical anisotropy was completely relaxed on immersion of the coal in pyridine. From changes in the optical anisotropy induced by pressure, the solvent-swollen coal was determined to be a cross-linked rubber and its macromolecular chain segments were found to have substantial mobility. It appears that pyridine and some other good swelling agents relax essentially all of the secondary bonding in the coal. On the other hand, raw coal and coal immersed in water, various hydrocarbon solvents and other poor swelling agents were determined to be macromolecular plastics.

More than half a century ago it was postulated that coal was a 'polymeric' or macromolecular substance¹⁻³. However, the evidence has not been completely convincing, and the particular nature of the macromolecular state has remained uncertain. Boddy⁴ found that coals, which normally exhibit brittle fracture, can apparently undergo plastic flow during crushing at room temperature under sufficiently high pressure if the particles are small enough. The results of crushing coal particles were reported to be, in general, the same for all coals including anthracites, even though anthracites possess a substantially higher degree of structural order than lower rank coals. Boddy also noted that fine mineral matter in the coal would spread and give translucent patches. In some of the coals, following plastic deformation, the flattened out coal would break up into small particles. Geoffrey⁵ has suggested that the plastic flow of coals under pressure might actually be the result of microfracturing followed by reconstitution of the particles by shearing forces. Newman⁶ in his work on plastic flow in coal has pointed out that even highly crystalline inorganic substances such as rock salt and corundum undergo plastic flow at sufficiently high hydrostatic pressures. In view of these behaviours, interpretation of the apparent plastic flow of the coal in terms of structure is uncertain.

Attempts have been made to measure the macromolecular parameters of coals. Sanada and Honda⁷ swelled coals in pyridine, which is a good swelling agent, and they utilized the statistical theory of rubber elasticity⁸ to calculate M_c (the average molecular weight between cross-links). However, these workers acknowledged that they could not justify application of this approach to coal. Indeed, the raw coal was not rubbery, and no definitive evidence that solvent-swollen coal has rubbery characteristics has been given. In addition, pieces of coal immersed in good swelling agents tend to fragment, so the application of thermodynamics was questionable. Investigators have also questioned whether the coal is chemically crosslinked⁹.

Larson and Kovacs¹⁰ calculated values for M_c using the stress-strain mechanical data on dry coal of Bangham and Maggs¹¹. The values calculated were very small and there was a large discrepancy between these values and those for M_c derived from solvent-swelling measurements. In a recent review of the molecular structure of coal, Davidson¹² concluded that 'a simple application of polymer theory is not justified' for determining macromolecular characteristics of coals.

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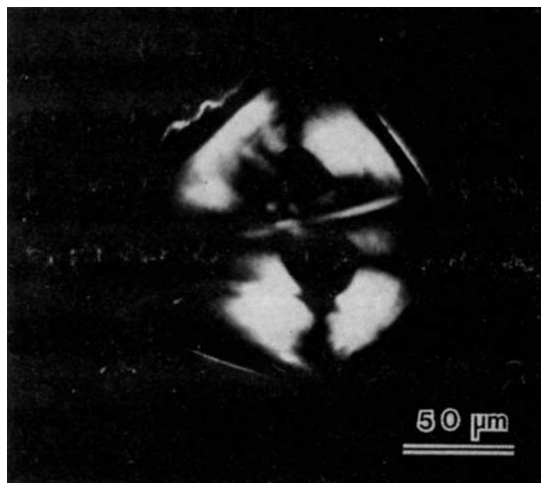


Fig. 1 Thin section sample of Illinois no. 6 coal immersed in pyridine and with pressure applied. It is viewed between crossed polarizers.

Some of the problems relating to the physical state of the coal were clarified by Brenner¹³ in his studies of the solvent-induced swelling of thin sections of a bituminous coal. It was found that when immersed in pyridine, the thin sections swelled in a regular manner and without much fracturing; and after the initial swelling, subsequent swelling and shrinkage was highly reversible. Therefore, the application of thermodynamics was reasonable. In addition, it was found that the swollen thin sections were highly flexible, indicating that the effective cross-link density was greatly decreased in the swollen state, or that the swollen sample was rubbery whereas the dry coal is glassy.

The results reported below give firm evidence that a bituminous coal swollen in a good swelling agent is a cross-linked rubber and that the dry coal is a macromolecular plastic. The specimens used in this study were uncontaminated thin sections of Illinois no. 6 coal, which is of high volatile bituminous rank. Since coals are highly heterogeneous, only pieces of the predominant (wood derived) component, vitrinite, were used. The samples were cut perpendicular to the bedding plane and ground to a thickness of about 15 μm (ref. 14). The samples were viewed between crossed polarizers in transmitted light on the stage of an optical microscope. It is well known that raw bituminous coals exhibit uniaxial optical anisotropy with the optic axis approximately perpendicular to the bedding plane¹⁵. Within a few seconds of placing a drop of pyridine on the sample, the optical anisotropy near the edges had disappeared. This behaviour is impressive because thermally the anisotropy is not destroyed until temperatures around 350 °C are reached. The rapid effect of the pyridine at room temperature indicates that the anisotropy or orientation in the raw coal is maintained strictly by secondary interactions.

A coverglass was placed on top of the thin section of coal immersed in pyridine. When pressure was applied, optical anisotropy was immediately produced in the sample. The anisotropy observed is quite unlike the original uniaxial pattern, having a roughly radial symmetry. Figure 1 shows a photomicrograph of a sample. A similar pattern of anisotropy is observed when pressure is applied on a rubbery polymer sheet¹⁶. Since no orientation was observable in the unstressed coal swollen in pyridine, and since it has been well established from extraction and degradation studies that coals contain extended organic molecules (or macromolecules), there can be little doubt that the anisotropy caused by application of stress results from an average orientation of the macromolecular chains. The rapidity of appearance of the anisotropy when the pressure is applied demonstrates that the macromolecular chain segments are highly mobile.

For the thin section of coal immersed in the pyridine, as long as pressure was maintained (for several hours), there was no noticeable change in the anisotropy. As the anisotropy was

maintained in spite of the mobility of the macromolecular chains, these chains must be connected to each other, or cross-linked, to prevent slippage and relaxation of the anisotropy. However, when the weight was removed, the anisotropy immediately disappeared. This rapid return to its undeformed state when stress is removed is characteristic of a rubber. Therefore, the solvent-swollen coal behaves like a cross-linked rubber.

The rubbery behaviour of small pieces of a thin section of coal swollen in the pyridine could also be observed without polarized light. When pressure was applied to the coverglass on top of the small pieces, they bulged out at the sides. When pressure was removed, they immediately retracted to their former size.

When the above studies were performed using *n*-propylamine rather than pyridine, similar results were obtained. It is expected that other very good swelling solvents for the coal will give similar results. On the other hand, when the coal was immersed in poor swelling agents such as water and various hydrocarbon solvents, no appreciable change in the natural uniaxial optical anisotropy occurred.

When the coal immersed in pyridine was allowed to dry without stress, it remained optically isotropic, for the most part. Therefore, it appears that the optical anisotropy of the raw coal is a metastable state locked in place by the secondary interactions. Once these interactions are relaxed, no memory remains to restore the uniaxial orientation. Pressure was applied to this dried, optically isotropic thin section. As pressure was increased, the sample began to spread out and optical anisotropy appeared and progressively increased in intensity in a roughly radial pattern. This induced anisotropy presumably results from a net orientation of the macromolecular chains—analogueous to the orientation produced previously by pressure on the solvent-swollen coal. When the pressure was removed from the dried thin section, the induced anisotropy remained. The continuous and regular formation of the radial anisotropy as pressure increased indicates a plastic flow mechanism (rather than, for example, microfracturing followed by reconstitution). The derivation and behaviour of this dried coal indicate that it behaves like a macromolecular glassy plastic.

When pressure was gradually applied to a thin section of raw coal, its optical anisotropy was observed to change progressively from the natural uniaxial pattern to a roughly radial anisotropy. Considering the relationship of the raw coal to the sample which had been swollen in pyridine and dried, the raw coal also behaves like a macromolecular glassy plastic. It is also likely that the natural uniaxial anisotropy of the raw coal results, at least in part, from a net orientation of the macromolecular chains.

The studies reported here investigated characteristics of a bituminous coal. Although coals vary considerably as a function of rank and other parameters, it is believed that the present findings will be relevant to a range of bituminous coals and probably also to some sub-bituminous coals.

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Plutonium redistribution by biological activity in Irish Sea sediments

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As part of a programme investigating the fate of transuranium nuclides discharged to coastal waters we have studied the effects of benthic animals on plutonium redistribution in Irish Sea sediments. From analyses of burrow linings of the large echiuroid *Maxmulleria lankesteri*, which penetrates to depths exceeding 40 cm, we now show that this animal may have a significant effect on the removal of plutonium to deeper layers in the sea bed.

Plutonium rapidly becomes associated with sedimentary particles when introduced into coastal marine environments^{1,2} and there has been considerable discussion as to the mechanisms and potential influence of plutonium post-depositional remobilization and redistribution¹⁻⁴. In particular, the role of biological mixing in transporting plutonium downwards^{5,6} and biologically-induced remobilization at depth, with subsequent upwards migration due to porewater advection⁵, have been considered. Plutonium discharged under authorization into the Irish Sea as part of the low-level liquid effluent from the British Nuclear Fuels Ltd reprocessing plant at Sellafield (UK) becomes incorporated into the sea bed to depths in excess of 30 cm. In many cases the isotopic composition of core profiles does not support the hypothesis that rapid sedimentation is solely responsible for the incorporation of plutonium into the sea bed⁷. The $^{239/240}\text{Pu}/^{238}\text{Pu}$ quotient in the Sellafield effluent before 1978 is not known precisely but from annual analyses of surface sediments it is believed to have fallen steadily from a value of about 20 in 1966 to a value of 4-5 since 1972¹. Thus the presence of a low quotient, at depth, indicates plutonium of more recent origin.

Bioturbation was considered to be a possible mechanism for plutonium migration. Williams *et al.*⁸ reported the presence in the sedimentary regime of the north-east Irish Sea of a large echiuroid, presumed to be *Amalosoma eddystonense*, and suggested it could be responsible for disturbing the sediment to a depth of at least 45 cm. Observations made by us in 1982 showed that this echiuroid, now correctly identified as *Maxmulleria lankesteri*, may be responsible for plutonium incorporation deep into the sediment. Little is known of the life history of *M. lankesteri*, indeed no male of this species has been recorded⁹. It is assumed by analogy with other echiuroids, especially *Echiurus echiurus*, that this species constructs and maintains a burrow by peristaltic action and mucous secretion and feeds by collecting surface sediment particles with its proboscis. From observations made of its burrow, defaecation occurs both at the surface and within the burrow. The shape of a complete burrow system is unknown. In May 1982 several fragments of this echiuroid species and parts of two of their presumed burrows were recovered from an area of fine mud lying off the Cumbrian coast using a Reineck box corer. The diameter of the burrows in relation to the size of the echiuroid and the presence of characteristic faecal pellets around and within the burrow, comparable with those pellets found in the echiuroid gut, were considered as evidence that both burrows had indeed been recently occupied by this species. A portion of the core containing the burrow was isolated with a 9-cm diameter plastic tube, extruded, and sectioned at 5-cm intervals. The sections were broken open and the brown-coloured burrow lining carefully

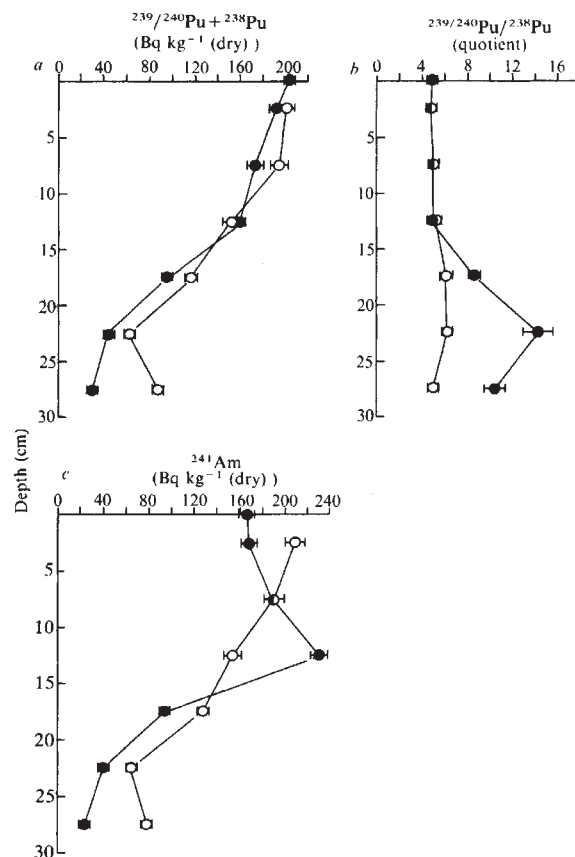


Fig. 1 Core 1 (54°19.0' N, 3°48.9' W), plutonium and americium distributions in burrow lining (○) and adjacent sediment (●). Horizontal bar represents $\pm 1\sigma$ counting propagated error.

removed. The adjacent grey sediment was also sampled, care being exercised to avoid both the burrow lining and the outer 0.5-cm annulus of the core. Samples were stored frozen before homogenizing and analysis for plutonium and americium by α spectrometry (Figs 1 and 2).

In core 1 the $^{239/240}\text{Pu} + ^{238}\text{Pu}$ concentration in both the burrow lining and the adjacent sediment decreased with depth (Fig. 1a) but the concentration in the former was consistently higher. The $^{239/240}\text{Pu}/^{238}\text{Pu}$ quotients were different, with lower values in the burrow lining than in the adjacent sediment at depth (Fig. 1b). This implies that, as well as having a higher plutonium concentration, the lining contained plutonium of more recent origin. The relatively undisturbed adjacent sediment also retained a sub-surface ^{241}Am maximum which was absent from the burrow lining (Fig. 1c). This accords with the marked decrease in ^{241}Am discharges since 1971-75 when discharges were 4-5 times higher than in previous years⁷. This evidence suggests that the burrow lining, at least in part, consisted of material derived from the sediment surface.

The data from core 2 (Fig. 2) do not fit into this pattern. The burrow lining has at least two components: (1) material representative of the sediment column at that depth but in contact with the overlying seawater, and hence plutonium of recent origin, through the burrow entrance; and (2) material actively transported up and down the burrow, particularly in the form of faecal pellets. The burrow lining of core 1 contained a large number of faecal pellets whereas material from core 2 had a much higher proportion of non-faecal, *in situ* material. It appears that the presence of faecal material is largely responsible for the observed increase in $^{239/240}\text{Pu} + ^{238}\text{Pu}$ and ^{241}Am concentration and decrease in $^{239/240}\text{Pu}/^{238}\text{Pu}$ quotient in the burrow lining of core 1 relative to the adjacent sediment. When a burrow is abandoned, other processes will continue to influence

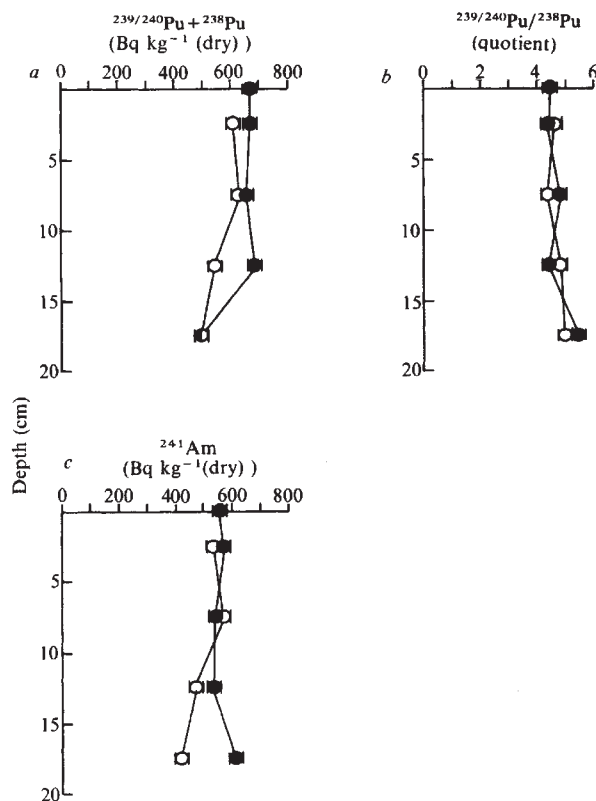


Fig. 2 Core 2 (54°21.9' N, 3°40.8' W), plutonium and americium distributions in burrow lining (○) and adjacent sediment (●). Horizontal bar represents $\pm 1\sigma$ counting propagated error.

plutonium behaviour: biodegradation and dispersal of faecal material, adsorption and desorption between the burrow lining and the adjacent sediment, and reworking by other organisms. The core 2 profiles may represent a later stage in the bioturbation process.

The animal can clearly bioturbate the sediment to a depth of at least 35 cm. Consequently the incorporation of plutonium and americium into soft sediments is affected to this depth. This fact is of considerable importance in the interpretation of core profiles obtained in this area. The extent of bioturbation will also affect estimates of the quantities and rates of sediment plutonium and americium remobilization into the water column. Further work in hand on the abundance and habits of *M. lankesteri*, and the other infauna of the area, will allow the significance of this mechanism for plutonium redistribution to be more fully assessed and quantified.

Thermal subsidence and eustasy in the Lower Palaeozoic miogeocline of western North America

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We have recently developed a procedure for calculating tectonic subsidence in fully-lithified basin sequences and applied it to Cambrian and Ordovician strata of the Cordilleran miogeocline exposed in the southern Canadian Rocky Mountains¹. The cumulative tectonic subsidence curves were found to be mainly thermal in form as predicted by the passive margin model for the miogeocline². In this paper we extend the analysis to Cambrian and Ordovician strata in a much larger segment of the miogeocline extending from the Yukon Territory to central Nevada. The results indicate that thermal contraction was the dominant subsidence mechanism for more than 2,000 km along the eastern or inner part of the miogeocline. In addition, we find a slight but persistent deviation in the subsidence curves from a purely thermal form that is consistent with a global sea level change proposed by Vail and others³ for Cambrian to middle Ordovician time.

Tectonic subsidence of a sedimentary basin is calculated by quantitatively removing the effects of sediment compaction, sediment loading, water depth changes and eustatic sea level changes (if known) from the cumulative thicknesses of the basin fill⁴. The procedure produces cumulative tectonic subsidence curves that can be compared with subsidence curves constructed from geophysical models of basin evolution⁵. To remove the effects of compaction and restore sedimentary layers in the miogeocline to original thicknesses and densities, we have applied empirical maximum and minimum delithification procedures to the miogeoclinal strata. The procedure is discussed fully in ref. 1 and only summarized here.

The maximum limit for delithification assumes that the thicknesses and densities of each stratigraphic unit change during burial solely by compaction. All lithologies in the units are fully compacted; that is, their porosities are essentially 0%. Hence, to calculate this limit, it is necessary to determine the average initial porosity and the change in average porosity during burial of each stratigraphic unit. This is done using an empirical porosity-depth curve for each of the most common lithologies in the stratigraphic units (solid curves in Fig. 1a).

To calculate the initial average porosity, the stratigraphic unit is placed on the porosity curves with its top at zero depth and its base at the depth equivalent to its present thickness. The porosity curves corresponding to each lithology in the unit are integrated from zero depth to the base of the unit, divided by the thickness of the unit and multiplied by the percentage of the corresponding lithology in the unit. The sum of the products is the average initial porosity. The thickness of the unit is then increased by the amount of water needed to fill the initial average porosity. The steps for calculating the initial average porosity are repeated using the new depth to the base of the unit until the difference in successive values for average initial porosity is less than 2%. The average initial density of the unit is calculated from the average grain density of the unit and its average initial porosity. To obtain the change in thickness and average density of the unit with burial, thickness and average porosity are calculated as above but using the successively deeper segments of the porosity curves that correspond to the increasing depths of burial of the unit as successively younger units are placed above it. This procedure is valid for compaction caused by purely mechanical movement of grains closer together and/or

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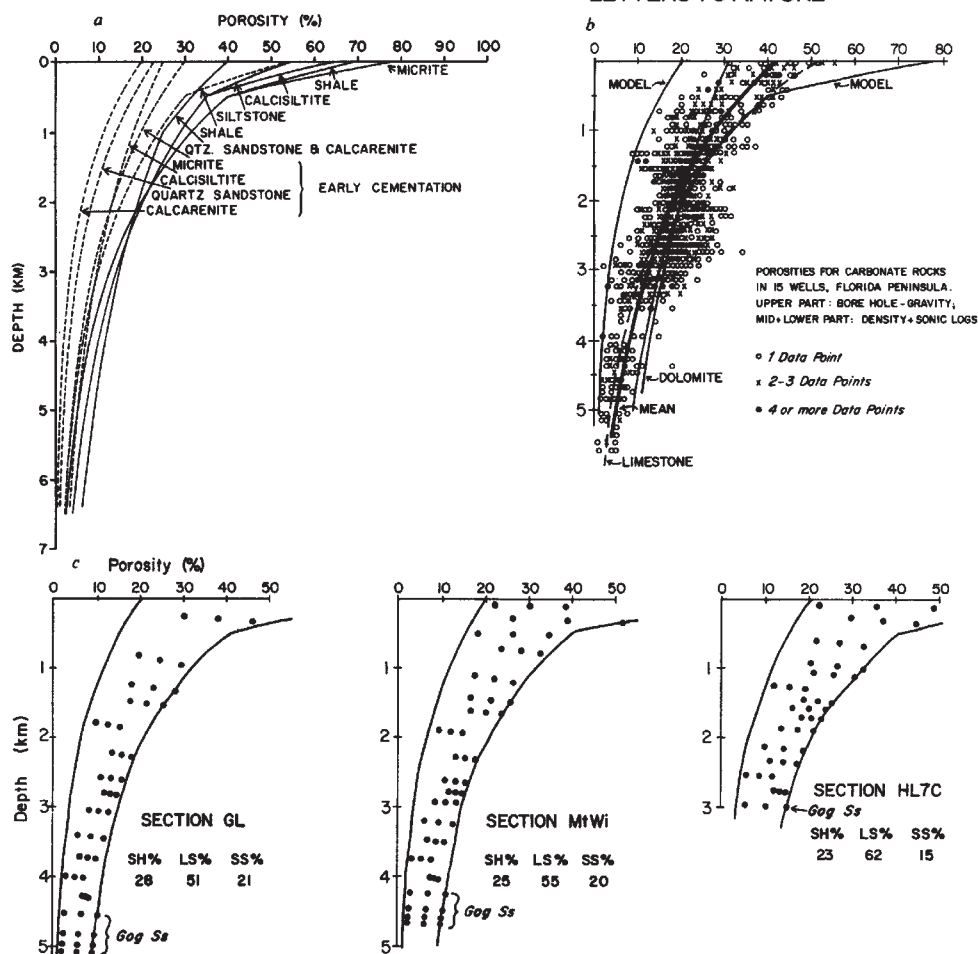


Fig. 1 *a*, Empirical porosity-depth curves used to delithify miogeoclinal stratigraphic sections. Solid curves are for maximum values of delithification; dashed curves are for minimum values. Construction of curves is based on published down-hole porosity-depth data from modern basins and experimental studies of compaction summarized in ref. 1. *b*, Porosity-depth values from wells in South Florida⁶ compared with limits (lines labelled model) of ranges of synthetic porosities in three miogeoclinal sections in *c*. *c*, Porosity-depth values calculated from the delithification procedures that were applied to three representative miogeoclinal sections. GL, MtWi and HL7C correspond to D₃, D₂ and E₂, respectively in Fig. 2*b* (figure from ref. 1).

by solution of the grains and reprecipitation of all dissolved material in nearby pore space.

The minimum limit for delithification assumes that only non-calcareous shales were compacted during burial and that the average initial porosities of all other lithologies were reduced solely by addition of a cement which originated from an external source and not from solution of grains within the section. The calculation of average initial porosity and change in porosity with burial is the same as for the maximum limit with two exceptions. The porosity curves used for the calculation are the dashed curves in Fig. 1*a*, and the present or measured thicknesses of each unit are increased only by the amount of water required to fill the porosity in the fraction of non-calcareous shale in the unit. The density of the cement that is added to fill the pore space is set equal to the average density; therefore, the calculation of the increase in density with burial is the same as for the maximum limit.

A test of the delithification procedures produced synthetic porosity-depth curves for the miogeocline (Fig. 1*c*) that correspond well to observed porosities in the modern margin off South Florida (Fig. 1*b*) which contains similar lithologies to those in the miogeocline. The misfit with low porosities between about

1.5 and 3.5 km in the wells of South Florida (Fig. 1*b*) occurs in lithologies composed mostly of dolomite⁶. However, delithifying dolomites in the miogeoclinal sequences using the best-fit exponential for dolomite determined by Schmoker and Halley⁶ (Fig. 1*b*), does not significantly change the values for delithification. We conclude that the maximum and minimum limits assumed for delithification are not unreasonable and an acceptable approximation to the true changes in sediment thicknesses and densities during burial lies between the limits.

For simplicity, we use an Airy compensation model to remove the effect of sediment loading and we ignore the effects of lateral heat flow. For margins that are more than about 100 km across these simplifications affect the slopes of the subsidence curves somewhat but do not distort their form^{1,7}. Palinspastic restorations of the miogeocline in the southern Canadian Rockies^{8,9} indicate that the ancient margin was more than 200 km wide. Radiometric ages for time-stratigraphic boundaries are from Armstrong¹⁰. We have found, however, that using other recently proposed time scales¹¹⁻¹⁴ does not distort the form of the subsidence curves.

Except for localities A and B (Fig. 2*a*), the Cambrian to Ordovician stratigraphic sequences selected for analysis are

Table 1 Thicknesses and ages for radiometrically dated intervals in miogeoclinal sections

Age	Section and thickness (m)															
	A	B	C ₃	C ₂	C ₁	D ₃	D ₂	D ₁	E ₂	E ₁	F ₂	F ₁	G	H	I	J
Early Camb.	963	540	1,833	1,250	455	752	580	448	296	257	352	302	961	1,167	767	1,738
Mid Camb.	432	150	1,514	1,136	1,093	1,545	1,269	1,218	1,132	1,062	978	852	962	894	715	873
Late Camb.	490	240	1,091	758	727	1,297	1,158	753	692	670	807	621	650	1,043	244	767
Tremadoc.		90	212	167	61	488	396	381	381	347	337	373				397
Arenig.						443	{398	{331	{356	{322	66	152	{428	{457	{244	{525
Llanv.													{95	{15		{192
Lland.							187		124	99						{457
Caradoc.							171		74							137

References to maps of areas above: A, 38; B, 39; C_{3,2,1}, 40; D₃, G.C.B.; D₂, 41 and 42; D₁, 43 and 44; E₂, 45; E₁, 46; F₂, 47; F₁, 48; G-J, 49; K, 49; M, 50.

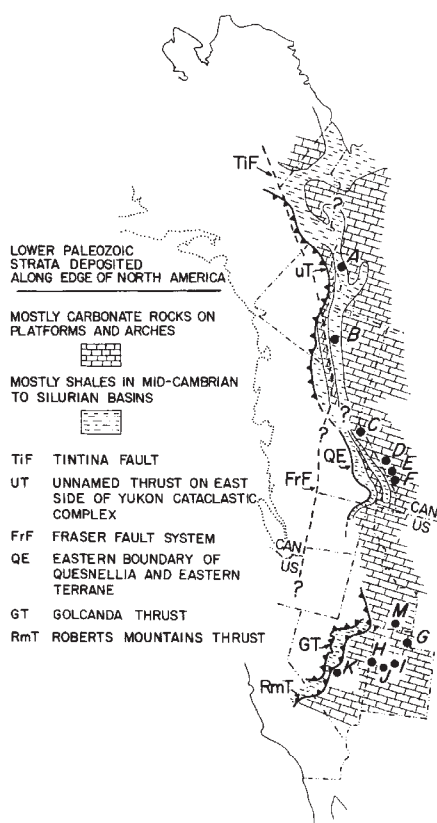
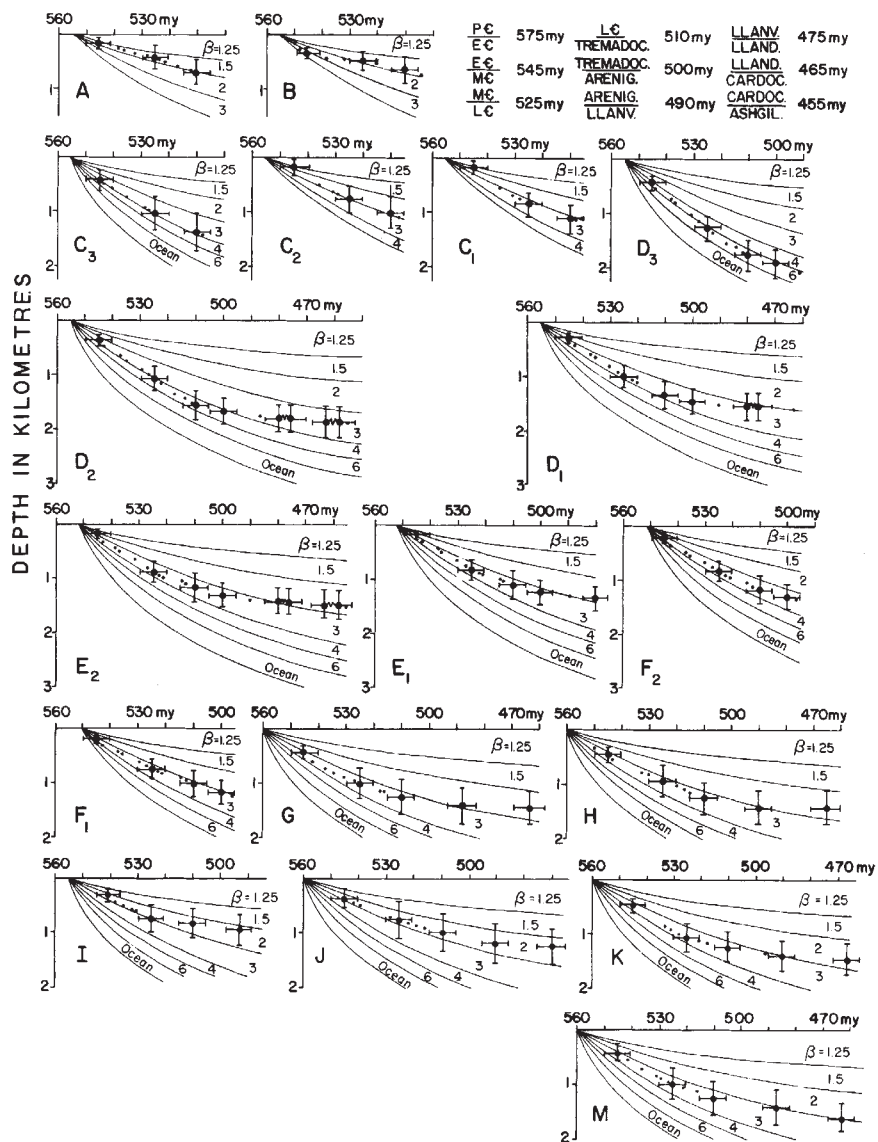


Fig. 2 a, Distribution of the lower Palaeozoic miogeoclinal facies in western North America. UT, QE, GT and RMT are major structural boundaries of various terranes accreted to western North America in post-early Palaeozoic time³¹⁻³³. These boundaries mark the westernmost preserved edge of the lower Palaeozoic North American continent. TIF-FrF is a major right-lateral transcurrent fault system^{26,34}. Filled circles give locations of sections in which tectonic subsidence has been calculated. Dotted outline of western North America gives position of this region after restoration of right-lateral transcurrent movement on the Tintina-Fraser fault system³⁴. b, Tectonic subsidence curves for sections located in Fig. 2a compared with thermal subsidence curves from the McKenzie stretching model¹⁹ (solid curves). Large dots are stratigraphic boundaries to which radiometric ages have been assigned. Small dots are formation boundaries positioned by assuming constant sedimentation rates. Vertical bars give range between maximum and minimum values of tectonic subsidence (see text). Horizontal bars give an assumed ± 5 Myr error for stratigraphic correlations with radiometrically dated boundaries. β = crustal stretching factors from McKenzie stretching model. Letters with subscripts designate different sections lying along east-west lines at locations that are too close to indicate separately in Fig. 1a. The higher subscript numbers designate the more westerly sections. Most of the sections with subscripts are more than 15 km apart on a non-restored base. Radiometric ages assigned to the stratigraphic boundaries are shown in the upper right-hand corner. Stratigraphic boundaries from Palmer³⁶



located within the easterly tapering wedge of mostly carbonate rocks that lies along the inner edge of the miogeocline. The great lateral extent and stability of depositional environments recorded by these strata indicates that they were deposited during the post-rift stage of the margin^{2,8,15}. Localities A and B are in the outer part of the miogeocline where, in places, complex block-faulting in Cambrian to Silurian time produced linear, shale-filled basins bounded by uplifted carbonate platforms¹⁶⁻¹⁸. Thicknesses, biostratigraphic ages and lithologies are from published measured sections (Table 1) except for E, F and D₁ where data are from detailed map cross-sections. Based on faunas and/or sedimentary structures, the strata in the sections accumulated within shelf environments¹⁵; hence, water depth corrections in the subsidence data are not necessary. Stratal loss at unconformities is relatively small (refs 15, 16 and G.C.B. and M.A.K., unpublished data) and should not have a significant effect on the form of the curves.

We have used two procedures to determine whether the subsidence curves are thermal in form. First, in Fig. 2b, the cumulative tectonic subsidence curves beginning at the base of

the middle Cambrian (545 Myr) are compared with post-rift thermal subsidence curves from the stretching model of McKenzie¹⁹ modified for a 5 km thickness of ocean crust^{7,20}. The age for T_0 , or the age of breakup and beginning of thermal contraction, is not well constrained from the geology, and we have positioned the tectonic subsidence curves by finding the best fit with the model curves. Second, in Fig. 3a the subsidence values are plotted against the $\sqrt{\text{time}}$ using the ages for T_0 given in Fig. 2b. We analyse the data in this form because thermal-mechanical models for margins imply that from about 16 Myr to 70 Myr after rifting, subsidence is a linear function of the $\sqrt{\text{time}}$ regardless of the rifting mechanism or the equilibrium thermal thickness of the lithosphere^{7,19,21-23}.

The overall form of the tectonic subsidence curves corresponds well to the shape of the thermal subsidence curves for the stretching model. In Fig. 3a, an approximation to a linear function of the $\sqrt{\text{time}}$ is indicated by the curves between about $3\sqrt{\text{Myr}}$ and $8\sqrt{\text{Myr}}$ (about 545 Myr and 475 Myr). The slopes of the subsidence curves (Figs 2b, 3a) are less than for subsidence of ocean floor²⁴ which is consistent with geological evidence

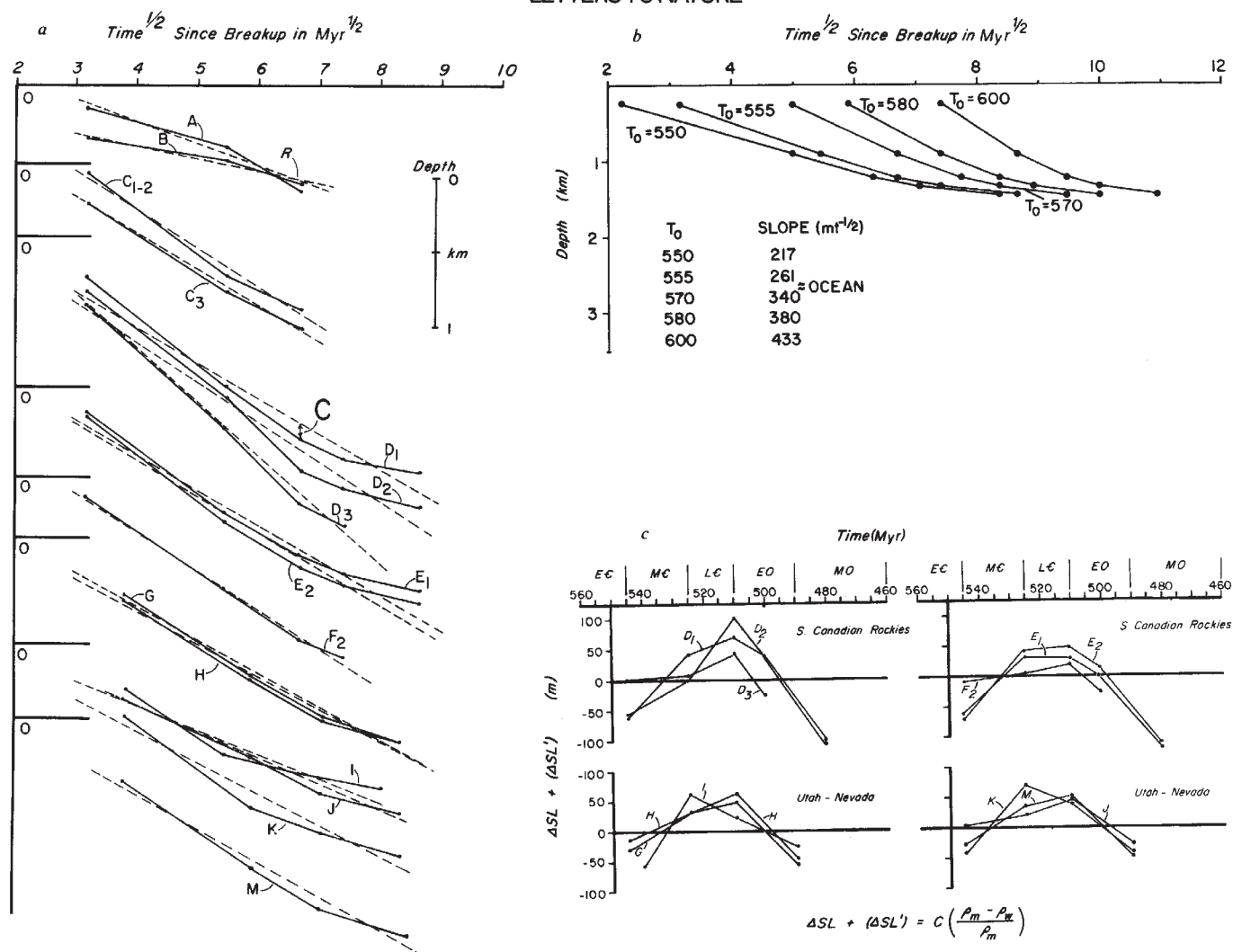


Fig. 3 *a*, Mid-points of the ranges between maximum and minimum values of tectonic subsidence in Fig. 1*b* plotted against $\sqrt{\text{time}}$. The values for T_0 are those indicated for the curves in Fig. 2*b*. Only data for the first 80 Myr after T_0 are considered in this analysis. Dashed lines (R) are lines fit by linear regression to the tectonic subsidence points. Correlation coefficients range from 1.0 (C_3 , D_3 , F_2) to 0.95 (A , D_1 , K). *b*, Change in form of subsidence curve as a result of assuming different values for T_0 , or age of breakup, in a typical section, E_2 , from the southern Canadian Rockies. The five solid points in the curves correspond to the first five large dots in the curve for E_2 , Fig. 2*b*. *c*, Calculation of apparent sea level change ($\Delta SL + \Delta SL'$) from the misfit, C, between tectonic subsidence curves and the lines fit by linear regression to subsidence points in Fig. 3*a*. $\Delta SL'$ is the difference between the 0 datum of the apparent sea level curves and present day sea level. The magnitude of ΔSL is not known and, therefore, the elevations of the apparent sea level curves should not be taken as the sea level change relative to present day sea level. ρ_M is density of mantle (3.40); ρ_W is density of sea water (1.03).

that the inner carbonate facies accumulated on continental crust^{9,25,26}. In addition, the large increases in depth of tectonic subsidence at certain localities (for example, D_1 to D_3 and C_1 to C_3) occur from east to west across restored distances of 60 to 80 km^{8,9}. Such changes are similar to increases in magnitudes of crustal thinning and tectonic subsidence over comparable distances from inner to outer parts of modern passive margins^{5,7}.

The ranges between the limits for the subsidence curves are not small (Fig. 2*b*), but those ranges are not strictly error bars. If delithification factors were specified for each of the units in a section, the form of the tectonic subsidence curve could fluctuate over the full length of the curve by about 40%, at most, of the distance between the limits, and the maximum variation that could occur between two successive points is about 15% of the distance between the limits. This is because once delithification factors are specified for a certain unit, lithification of the strata in that unit no longer contributes to the calculation of the maximum and minimum limits at the time of deposition of the higher units in the section. The tectonic subsidence curve can only lie along the minimum or maximum limit or somewhere between the two limits and nearly parallel to them. Thus, regardless of which delithification factors are applied to the lithologies,

the form of the tectonic subsidence curves would be essentially the same. Considered together, the subsidence data in Fig. 2*b* and 3*a* argue for a thermal subsidence mechanism within the miogeocline from northern British Columbia to central Nevada. The thermal form of subsidence on this large, essentially continental scale, is compelling geophysical evidence in support of the passive margin model for the Cordilleran miogeocline.

In Fig. 3*b*, the forms of the curves for different values of T_0 , or age of breakup, in a representative section, E_2 , indicate that values of T_0 of about 580 Myr and greater yield a distinctly curvilinear form with the $\sqrt{\text{time}}$ and produce average rates of subsidence that are greater than those observed for modern ocean floor²⁴. It would seem unlikely that the age of breakup and beginning of thermal contraction could be older than, at most, 600 Myr. The age of 550 Myr to 600 Myr we have previously suggested for the age of breakup in a small segment of the passive margin in the southern Canadian Rockies¹ (locations D, E, F) appears to be appropriate for a much larger part, and possibly all, of the miogeocline. It would seem that a single continent or continental fragment with a rifted edge more than 2,000 km in length broke away from western North America close to the time of the Precambrian-Cambrian boundary. The rifted piece could still be intact somewhere on the globe;

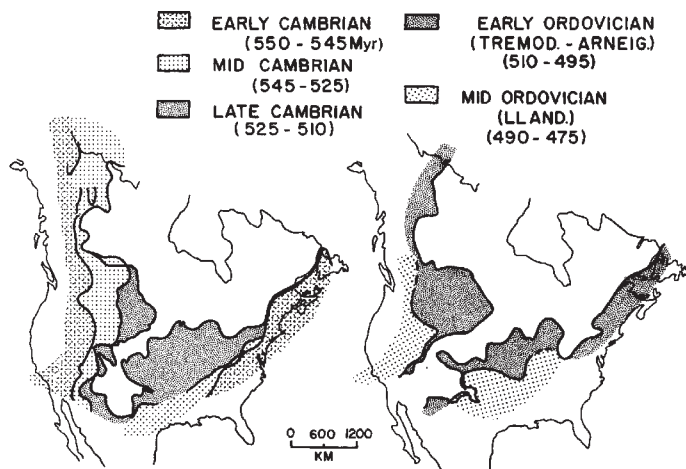


Fig. 4 Preserved early Cambrian to middle Ordovician strata in North America³⁷ indicating the timing and magnitude of the early Palaeozoic transgression and regression. Note the similarity in timing to the form of the early Palaeozoic apparent sea level curves in Fig. 3c.

knowledge of its size, location and late Proterozoic to early Palaeozoic history obviously would be of considerable importance to the tectonic evolution of the North American Cordillera.

A slight deviation from a purely thermal form (or a linear function of the $\sqrt{\text{time}}$) in about the first 70 Myr (Figs 2b, 3a) occurs in most of the sections at essentially the same time, suggesting a subtle but widespread event superimposed on the thermal subsidence in the margin. The position of the misfit in the $\sqrt{\text{time}}$ segments of the curves makes it unlikely that it results from an inappropriate stretching model or an incorrect assumption for the thickness of the lithosphere. Among the possible explanations are a minor lithospheric heating event that retarded cooling of the margin during late Cambrian to Ordovician time, a reduction in the rate of cooling due to the insulating effect of the sediment blanket, or an eustatic rise and fall of sea level. A test of the first two requires much additional data and modelling. An approximation to an eustatic change that would account for the misfit, however, can be easily calculated from the available data assuming an Airy-type of response to water loading and unloading²⁷. We calculate values for the apparent eustatic change from the difference (C) between a tectonic subsidence- $\sqrt{\text{time}}$ plot and a straight line fit by linear regression to the tectonic subsidence values (Fig. 3a).

The results of the calculation (Fig. 3c) are suggestive of an eustatic mechanism in two respects. First, the apparent sea level change has approximately the same timing and amplitude in 12 localities extending over a distance of more than 1,500 km. Secondly, the timing of the apparent sea level change is consistent with the well-documented middle Cambrian to late Cambrian or earliest Ordovician transgression and early to middle Ordovician regression on the North American craton^{28,29} (Fig. 4), an event which Vail and others³ suggest is synchronous on a world-wide scale and a result of eustasy. The maximum extent of the transgression at late Cambrian or earliest Ordovician time is over 600 km inland from the miogeoclines (passive margins) rimming most of the North American continent²⁸ (Fig. 4). This extent clearly exceeds the landward limit of flexurally controlled subsidence (up to about 250 km) that has been suggested to account in part for transgressions onto cratons adjacent to subsiding Mesozoic and Cenozoic passive margins³⁰. We suggest that an eustatic rise and fall of sea level is a strong possibility for Cambro-Ordovician time and should be further tested, especially with analyses of subsidence in other lower Palaeozoic miogeoclines.

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The cranial venous sinus system in *Australopithecus afarensis*

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An enlarged occipital-marginal venous sinus system occurs in much higher frequencies in cranial remains of robust australopithecines and *Australopithecus afarensis* than in crania representing other fossil or extant hominids. A detailed functional interpretation of this 'accessory' sinus system is suggested here. Such a system would have permitted blood to flow preferentially to either the vertebral or the internal jugular system,

depending on postural and respiratory changes, and thus provides a unique solution to the increased circulatory demands on the vertebral venous plexus associated with upright posture and bipedalism. The distribution of this trait among fossil hominids suggests that *A. afarensis* was directly ancestral to, or shared a common ancestor with, robust australopithecines.

In his classic study of *Zinjanthropus*, Tobias^{1,2} described an interesting variation of the cranial venous sinus system that characterized this robust australopithecine. This specimen of *Australopithecus boisei* had a reduced transverse-sigmoid sinus complex and an unusually enlarged occipital-marginal (accessory) sinus complex for draining intracranial structures (Fig. 1). Such an enlarged accessory complex is absent in all undoubted gracile australopithecine and *Homo habilis* specimens in which the pertinent anatomy is preserved¹⁻³. This cerebral venous drainage morphology is also rare in modern pongids (usually less than 1%) and unusual in *Homo sapiens* (usually <10%)^{1,4,5}. By way of contrast, all undoubted *A. robustus/boisei* specimens recovered to date and preserving the relevant anatomy (Table 1) show this enlarged accessory sinus complex^{1,6-9}.

We view the high frequency of this endocranial trait as a specialization (apomorph) of the robust australopithecine lineage that distinguishes these populations from those of gracile australopithecines, early *Homo* and extant hominoids (including man). We do not believe the frequency distribution of this trait in the robust lineage can be accounted for by random sampling bias. For example, if we assume that the robust australopithecine sample is drawn from a population with a frequency of this trait equivalent to that of modern human populations (~10%), then the theoretical binomial probability of randomly selecting six skulls all having this trait is 0.000001. If the frequency of the trait approached the highest limits known for some local *Homo sapiens* populations (~25%, W. Kimbel, personal communication), then the probability of recovering six such skulls is still only 0.00024, that is, virtually inconceivable. It thus appears certain that the *robustus/boisei* populations had a significantly higher frequency of the specialized morphotype than is found in any other known hominoid population, living or extinct.

This specialized morphotype also typifies another group of early hominids that predate the robust australopithecines listed in Table 1, namely, *A. afarensis*. All four *A. afarensis* specimens from Hadar represented by the appropriate area of the occiput are described as having enlarged portions of this accessory sinus system¹⁰.

If the high frequency of this morphotype is distinctive of the robust lineage, then its presence in the four *A. afarensis* specimens from Hadar has obvious phylogenetic and systematic significance. While we urge caution in erecting new phylogenetic schemes, it is important to note that Olson¹¹ quite independently came to the conclusion that *A. afarensis* specimen AL-333-45 from Hadar was the earliest known member of the robust australopithecine lineage based on his study of its basicranial anatomy. In light of Olson's and our findings, it is interesting that Johanson was the first to remark on the possible presence of 'an early occurrence of a robust australopithecine lineage in the Hadar population'¹²⁻¹⁴. Note also that functional interpretation of the enlarged accessory sinus system (below) suggests that some (if not all) of the Hadar australopithecines might represent a population that is ancestral to later robust australopithecine populations. The view¹⁵ that *A. afarensis* (with an enlarged accessory complex) gave rise to *A. africanus* (with a modern looking transverse-sigmoid complex) which in turn gave rise to *A. robustus/boisei* (again with an enlarged accessory complex) is therefore unlikely.

There is considerable variation in the venous sinuses that converge at the confluence of sinuses (torcular herophili)^{4,5,16-18}. The causative factors influencing the asymmetrical blood flow in this region are still the subject of debate¹⁹ but we believe that the specialized morphotype found in the robust lineage is a specialized solution to the increased circulatory demands on

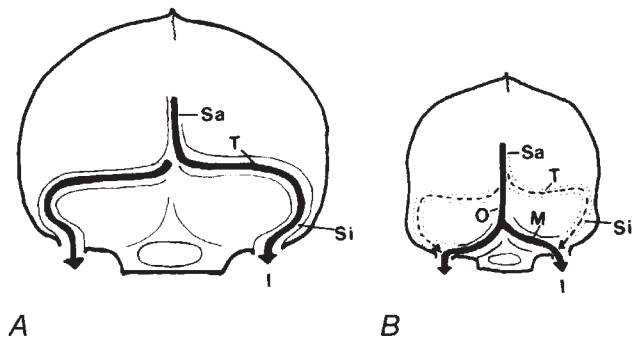


Fig. 1 Occipital views of typical cranial venous sinus systems in modern man (A) and fossil robust australopithecines and *A. afarensis* (B). In A, blood flows from the superior sagittal sinus (and the straight sinus from within) to the transverse-sigmoid sinuses and then exits the cranium via the internal jugular veins. The transverse-sigmoid system is greatly reduced or missing in B, in which a large portion of blood is drained to the internal jugular vein and the vertebral plexus (see Fig. 2) through an enlarged occipital-marginal system. Abbreviations: I, internal jugular vein; M, marginal sinus; O, occipital sinus; Sa, superior sagittal sinus; Si, sigmoid sinus; T, transverse sinus.

the vertebral venous plexus that are associated with upright posture and bipedalism (see below).

A small accessory sinus system is typically present in man²⁰⁻²³. This accounts for the fact that Das and Hasan⁵ were able to discern this system in 65% of 200 cadavers (although only about 6% of cases had a morphotype similar to the *robustus/boisei* pattern). During embryological development, an occipital sinus extends caudally from the tentorial venous plexus while a marginal sinus develops rostrally from the posterior venous plexus^{24,25}. As the cerebrum expands caudally the transverse sinus also moves in that direction and the occipital and marginal sinuses are brought closer together. The two sinuses join during late fetal development to complete the accessory sinus system^{21,22}. Several authors have commented on the relatively large size of the accessory sinus system in human fetuses and neonates^{4,18,26} while others^{21,22,27} have illustrated this system as somewhat larger in the infant than in the adult. However, these observations are in need of quantification and discussion in terms of allometry and functional significance (for example, the occurrence of this morphology in neonates appears before the secondary cervical curve forms, that is, before the head is held upright by the infant).

Table 1 reveals an interesting asymmetry in venous flow. In the four specimens for whom evidence is available bilaterally, the accessory sinus system is larger on the right side. Since right-sided dominance in cerebral venous flow is frequently present in modern man and in many non-human primates²⁸⁻³¹, it may be that cerebral venous drainage dominance has been shifted (lowered) from the transverse-sigmoid to the accessory system in the robust lineage.

In human neonates and adults the accessory sinus system drains into both the internal jugular and, especially, the vertebral plexus through numerous small channels around the foramen magnum^{5,20,32-34} (Fig. 2). This is important since the vertebral venous plexus is a *major* channel of cerebral venous drainage in an upright posture^{34,35}.

In man the vertebral plexus is a valveless, extensive, anastomotic network stretching from pelvis to cranium. There is a direct relationship between vertebral venous pressure and cerebrospinal pressure along the axial skeleton. For example, in a supine individual (or quadruped) the cerebrospinal fluid (CSF) pressure in the lumbar subarachnoid space equals the pressure in the superior jugular bulb (about 10 cm H₂O). However, a change in body position to an upright posture produces considerable hydrostatic pressure changes such that the occipital CSF pressure becomes subatmospheric (around -5 cm H₂O) and the lumbar CSF pressure rises to over

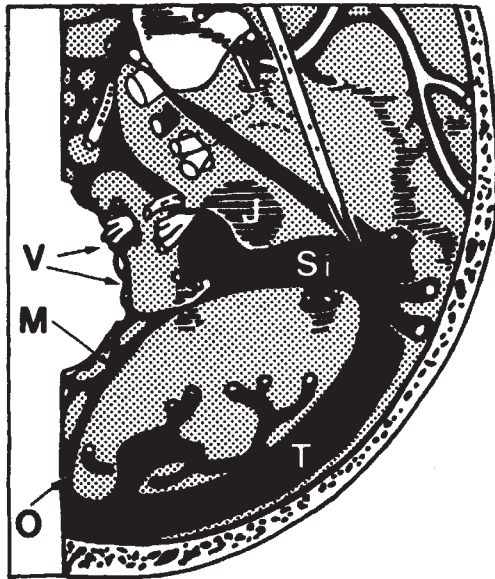


Fig. 2 The occipital-marginal sinus system in adult humans; right horizontal section after Padgett²². Note numerous connections with the internal vertebral plexus. This system is usually small in man; its enlargement would permit a quantity of blood to flow preferentially to either the vertebral plexus or the internal jugular vein, depending on momentary postural and respiratory constraints. Abbreviations: J, jugular bulb; V, internal vertebral plexus; others, see Fig. 1.

40 cm H₂O. This negative pressure can only be maintained because the dural sinuses and internal vertebral plexus are protected by bone from collapsing; the result is that they function as a siphon to aid in perfusion of the brain. In the supine position the blood is delivered to the brain at a pressure close to 85 mm Hg but in the upright position this arterial pressure is lower due in part to the height of the brain above the heart³⁵. This drop in arterial pressure is compensated for by the lowering of cerebral venous pressure to the subatmospheric levels mentioned above—thus, in an upright posture, under conditions of increased intrathoracic pressure such as during normal expiration or heavy lifting (forced expiration), intracranial venous blood drains preferentially to the lower pressure regions, that is, the vertebral plexus. On the other hand, when intrathoracic pressure is low during upright posture, such as during inspiration, blood tends to flow through the internal jugular system³⁵. Thus, a key feature of the venous circulatory system associated with bipedalism is the ability of blood to flow to either the vertebral or the internal jugular system, depending on momentary respiratory and postural changes.

One can suggest likely hypotheses concerning cerebral venous drainage in early hominids from the data provided above. It seems likely that at least small accessory sinuses were present in the earliest hominids since they are a common feature in bigger brained anthropoids including hominoids^{24,25,28-30}. For physiological reasons, it is also reasonable to suggest that selection for bipedalism placed an increasing cerebral venous drainage load on the vertebral plexus. By 3 Myr ago, early robust-like hominids¹¹ had already responded to this selection pressure by developing to a very high frequency the enlarged accessory sinus system which has numerous connections to the vertebral plexus. Such a system is seen in *A. afarensis*, *A. robustus* and *A. boisei*. The way in which other bipedal hominids such as *A. africanus* and *H. habilis* responded to similar selection pressures is currently under investigation by the authors.

Table 1 Enlarged accessory sinuses in robust australopithecines and fossil hominids from the Hadar Formation, Ethiopia

Specimen	Ref.	Accessory sinus
Robust australopithecines		
OH 5	1, 14	⊗
ER 407	6	⊗
ER 732	7	⊗
SK 1585	8	⊗
SK 859	1, 14	⊗
KNM-CH 304	9	⊗
Hadar hominids		
A.L. 333-45	10	⊗
A.L. 333-105	10	⊗
A.L. 333-114	10	⊗
A.L. 333-116	10	⊗

Diagrams in the right-hand column show available portions of the occiput. Whole circles indicate that enough of the appropriate area was available to determine state of sinus on both sides; half circles signify that the appropriate region is fragmentary or lacking on one side. Note right dominance (enlargement) of marginal sinus in the four specimens that are represented bilaterally. Solid line diagrams represent specimens whose endocasts have been studied by one of us (D.F.) or, in the case of SK 859, a specimen whose sinus system has been illustrated in the literature. Remaining diagrams (dashed) are based on unillustrated descriptions from the literature.

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Melatonin is a potent modulator of dopamine release in the retina

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Melatonin, a hormone originally discovered in the pineal gland¹, has also been found in the retina of several vertebrate species²⁻⁵. The enzyme system for melatonin synthesis also exists in the retina^{2,3,6-8}, where the activity of one such enzyme, (serotonin *N*-acetyltransferase) varies with changes in light intensity in a circadian pattern^{3,8}. As the activity of dopamine containing amacrine neurones of the retina is influenced by changes in illumination⁹⁻¹² it was of interest to determine the effect of melatonin and its precursors, serotonin and *N*-acetylserotonin, on the release of ³H-dopamine from rabbit retina. I report here that picomolar concentrations of melatonin (*IC*₅₀ 9 pM) selectively inhibited the calcium-dependent release of ³H-dopamine from rabbit retina, but not from striatum. Melatonin, was 1,000 times more potent than its precursor *N*-acetylserotonin in inhibiting the release of ³H-dopamine in retina, while the putative neurotransmitter serotonin¹³, was inactive. It is suggested that the light-dependent production of melatonin could play a physiological role in modulating the activity of dopamine-containing neurones in the retina.

Albino rabbits (2-3 kg) maintained on 14-10 h light-dark cycle, were killed by decapitation about the middle of the light cycle. Retinas were dissected free of pigmented epithelium and prepared for superfusion as described previously^{14,15}. All experimental procedures were carried out in conditions of illumination. The pieces of rabbit retina or striatal slices (0.4 mm) were labelled *in vitro* with 0.1 μ M ³H-dopamine (specific activity 28.4 Ci mmol⁻¹) for 20 min in Krebs' solution at 37°C. Thereafter, the retina pieces or striatal slices were superfused with Krebs' solution (calcium concentration 1.3 mM) until the spontaneous outflow of radioactivity had levelled off. Tritium release was elicited twice in each experiment either by field stimulation at 3 Hz for 1 min (20 mA, 2 ms duration) or by exposure to 1 μ M tyramine for 2 min. The first (*S*₁) and second (*S*₂) periods of stimulation were applied 60 min and 100 min after the end of the incubation with ³H-dopamine respectively. Four-minute samples of the superfusate were collected before, during and after the period of stimulation and the tritium released determined. Results were calculated as the percentage of the total tissue radioactivity released in each sample^{14,15}.

Field stimulation releases ³H-dopamine previously taken up by rabbit retina pieces through a calcium-dependent process^{14,15}. In the controls, the spontaneous outflow expressed as the percentage of total tissue radioactivity released during the 4-min preceding the first period of stimulation (*S*₁) was 1.65 $\pm$ 0.13% (*n* = 8). The increase above the spontaneous levels, of the total tissue radioactivity released by the first 1-min period of stimulation (*S*₁) at 3 Hz was 1.49 $\pm$ 0.21% (*n* = 8) and after the second period of stimulation (*S*₂) was 1.56 $\pm$ 0.20% (*n* = 8). The ratio obtained between the two consecutive periods of stimulation, in the absence of drugs (*S*₂/*S*₁) was 1.06 $\pm$ 0.04 (*n* = 8), (Fig. 1A). The putative neurotransmitter, serotonin (0.1 nM-1 μ M), did not affect either the spontaneous or field stimulation-evoked release of ³H-dopamine from rabbit retina (Fig. 1). Concentrations of serotonin higher than 1 μ M significantly increased the spontaneous outflow of radioactivity, through a calcium-independent process (unpublished observations). Figure 1A shows that the active metabolites of serotonin, *N*-acetylserotonin (1 nM-1 μ M) and melatonin (1 pM-1 μ M)

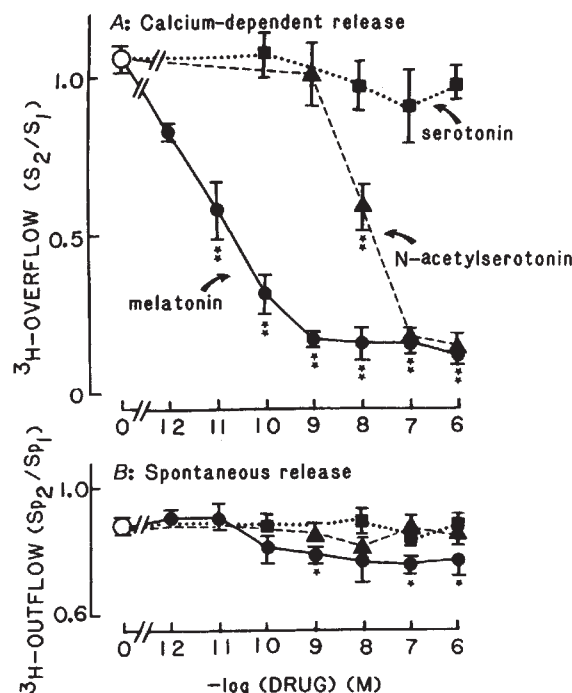


Fig. 1 Effect of serotonin, *N*-acetylserotonin and melatonin on the spontaneous and calcium-dependent release of ³H-dopamine from rabbit retina. Ordinates: A, ³H-overflow, is the percentage of total tissue radioactivity released by field stimulation above the spontaneous levels of release. Results are expressed as the ratio obtained between the second (*S*₂) and the first (*S*₁) stimulation periods within the same experiment. The calcium-dependent release of ³H-dopamine was elicited by a 1-min period of field stimulation at 3 Hz (20 mA, 2 ms). In the controls, the percentage of the total tissue radioactivity released after the first 1-min period of stimulation (*S*₁) was 1.49 $\pm$ 0.21% (*n* = 8). The radioactivity retained by the control tissue after 120 min superfusion was: 47.3 $\pm$ 4.8, nCi per chamber (*n* = 8). B, ³H-outflow, is the percentage of total tissue radioactivity release in the four-min sample preceding the second (*S*₂) and the first (*S*₁) period of field stimulation, expressed as the ratio *S*₂/*S*₁. In the controls, the spontaneous outflow calculated as the percentage of total tissue radioactivity released during the 4-min preceding the first period of stimulation (*S*₁) was 1.65 $\pm$ 0.13% (*n* = 8) and the second stimulation (*S*₂) was 1.17 $\pm$ 0.14% (*n* = 8). The ratio *S*₂/*S*₁ was: 0.88 $\pm$ 0.02. Abscissae: Molar concentrations of the drugs (logarithmic scale). Drugs were added 20 min before *S*₂. Only one concentration of each drug was tested per experiment. $\circ$, control; $\bullet$, melatonin; $\blacktriangle$, *N*-acetylserotonin; $\blacksquare$, serotonin. Shown are mean values $\pm$ s.e.m. of 3 to 8 experiments per group. **P* < 0.05; ***P* < 0.001 when compared with the corresponding control, (Student's two-tailed *t*-test).

inhibited in a concentration-dependent manner the calcium-dependent release of ³H-dopamine from the retina. The concentration of melatonin inhibiting the release of ³H-dopamine by 50% (*IC*₅₀) was 9 pM, being 1,000 times more potent than its precursor *N*-acetylserotonin (*IC*₅₀ 8.6 nM). *N*-Acetylserotonin at 0.1 μ M and melatonin at 1 nM maximally inhibited the calcium-dependent release of ³H-dopamine by 80% of the control value (Fig. 1A). This inhibitory effect of melatonin was almost fully reversed by superfusing the retina with Krebs' solution free of melatonin for 20 min before a third period of stimulation (data not shown). It is of interest that the concentrations of the most potent dopamine receptor agonist¹⁶ (pergolide, *IC*₅₀ 5 nM), opiate receptor agonist¹⁵ (D-Ala²,Met⁵-enkephalinamide, *IC*₅₀ 30 nM) or α -receptor agonist¹⁷ (clonidine, *IC*₅₀ 56 nM), necessary to inhibit the release of ³H-dopamine from retina by 50% are 500-6,000 times higher than the concentration of melatonin required to produce the same effect.

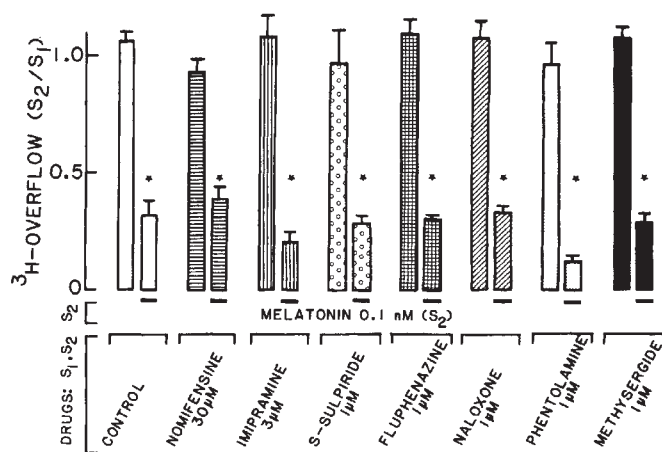


Fig. 2 Effects of neuronal uptake inhibitors and various receptor antagonists on melatonin-induced inhibition of ^3H -dopamine release. Ordinate: ^3H -overflow, is the percentage of total tissue radioactivity released by field stimulation above the spontaneous levels of release. Results are expressed as the ratio obtained between the second (S_2) and the first (S_1) stimulation periods within the same experiment. Release was elicited by field stimulation for 1 min at 3 Hz (20 mA, 2 ms). The neuronal uptake inhibitors or the receptor antagonists were added to the medium 40 min before S_1 and maintained until the end of the experiment. The percentage of total tissue radioactivity released during the first period of field stimulation (S_1) was $1.49 \pm 0.21\%$ ($n=8$), in controls; $4.41 \pm 0.62\%$ ($n=4$), with $30 \mu\text{M}$ nomifensine; $1.78 \pm 0.09\%$ ($n=3$), with $3 \mu\text{M}$ imipramine; $2.86 \pm 0.3\%$ ($n=6$), with $1 \mu\text{M}$ S-sulpiride; $2.10 \pm 0.50\%$ ($n=4$), with $1 \mu\text{M}$ fluphenazine; $1.71 \pm 0.29\%$ ($n=4$), with $1 \mu\text{M}$ (-)naloxone; $2.06 \pm 0.24\%$ ($n=4$), with $1 \mu\text{M}$ phentolamine; and $2.61 \pm 0.46\%$ ($n=4$), with $1 \mu\text{M}$ methysergide. Melatonin 0.1 nM , was added 20 min before the second period of stimulation (S_2) only in the groups indicated with a black bar (—). Only one concentration of melatonin was tested per experiment. Shown are mean values $\pm$ s.e.m. of 3 to 8 experiments per group. * $P < 0.001$ when compared with the corresponding control, (Student's two-tailed t -test).

The effect of melatonin on the release of radioactivity elicited by the indirect amine tyramine was also investigated. Total tissue radioactivity released above the spontaneous levels by the first 2-min period of exposure to $1 \mu\text{M}$ tyramine (S_1) was $3.62 \pm 0.22\%$ ($n=4$), and after the second period of stimulation with tyramine (S_2) was $3.65 \pm 0.40\%$, $n=4$. The release of radioactivity elicited by tyramine from the retina is mediated through a calcium-independent process since omission of calcium did not affect this release. A concentration of melatonin (0.1 nM), which inhibited the calcium-dependent release of ^3H -dopamine by 70% (Fig. 1A), failed to modify the increase in radioactivity elicited by tyramine [$S_2 = 4.17 \pm 0.36\%$ ($n=3$), obtained in the presence of 0.1 nM melatonin]. The spontaneous outflow of radioactivity, which is also a calcium-independent process¹⁴, was only slightly inhibited by concentrations of melatonin higher than 1 nM (Fig. 1B). These results suggest that melatonin inhibits only the calcium-dependent release of ^3H -dopamine from the rabbit retina.

The inhibitory effect of melatonin on the depolarization-evoked ^3H -dopamine release appears to be selective for the retina, since this hormone failed to modify the calcium-dependent release of ^3H -dopamine from other structures with dense dopaminergic innervation such as the striatum (Table 1) and olfactory tubercle (unpublished observations). Similarly, Zisapel and colleagues^{18,19} reported that melatonin inhibited the calcium-dependent release of ^3H -catecholamines from slices of rat preoptic and hypothalamic areas previously labelled with ^3H -dopamine, but not from striatal slices. Table 1 also shows no inhibitory effect of N -acetylserotonin ($0.1 \mu\text{M}$) or serotonin ($0.1 \mu\text{M}$) on ^3H -dopamine release from the striatum. On the contrary, under identical experimental conditions, the dopamine receptor antagonist apomorphine ($0.1 \mu\text{M}$) induced a significant

Table 1 Lack of effect of serotonin, N -acetylserotonin and melatonin on the calcium-dependent release of ^3H -dopamine from rabbit striatum

Drugs in S_2	n	^3H -Transmitter overflow		Ratio† S_2/S_1
		% Total tissue radioactivity*	S_2	
Control	6	2.09 ± 0.26	2.17 ± 0.25	1.05 ± 0.03
Serotonin $0.1 \mu\text{M}$	7	2.80 ± 0.26	3.01 ± 0.15	1.13 ± 0.11
N -acetylserotonin $0.1 \mu\text{M}$	7	2.84 ± 0.43	3.10 ± 0.31	1.16 ± 0.09
Melatonin $0.1 \mu\text{M}$	4	2.55 ± 0.52	2.69 ± 0.45	1.07 ± 0.05
Apomorphine $0.1 \mu\text{M}$	4	2.19 ± 0.87	$0.86 \pm 0.46^\ddagger$	$0.35 \pm 0.07^\S$

Drugs in the concentrations indicated were added to the medium 20 min before S_2 . In the controls, the spontaneous outflow of radioactivity calculated as the percentage of total tissue radioactivity released during the 4-min period preceding the first period of stimulation (S_1) was: $0.92 \pm 0.06\%$ ($n=6$) and the second stimulation (S_2) was: $0.96 \pm 0.04\%$ ($n=6$). The ratio S_2/S_1 was: 0.95 ± 0.03 ($n=6$). Neither serotonin, N -acetylserotonin, melatonin nor apomorphine modified the spontaneous outflow of radioactivity at the concentration indicated when added 20 min before the second stimulation (S_2). The radioactivity retained by the control tissue after 120 min of superfusion was: $88.05 \pm 7.52 \text{ nCi}$ per slice, $n=6$. Shown are mean values $\pm$ s.e.m.; n =no of experiments per group.

*Percentage of the total tissue radioactivity released above the spontaneous levels by 1-min period field stimulation at 3 Hz (20 mA, 2 ms). The interval between two consecutive periods of stimulation (S_1 , S_2) was 40 min.

†Ratio of the percentage of total tissue radioactivity released during the second stimulation (S_2) to that released during the first stimulation period (S_1).

‡ $P < 0.05$; § $P < 0.001$, when compared with control (Student's two-tailed t -test).

decrease in ^3H -dopamine release (Table 1), probably through activation of inhibitory dopamine autoreceptors¹⁴.

The inhibition of ^3H -dopamine release by 0.1 nM melatonin was not modified in the presence of inhibitors of dopamine uptake (nomifensine, $30 \mu\text{M}$) and serotonin uptake (imipramine, $3 \mu\text{M}$) (Fig. 2). These results suggest that the decrease in ^3H -dopamine release elicited by melatonin is not due to an increase in the neuronal uptake of monoamines. Furthermore it is unlikely that melatonin is interacting with retinal D-2 dopamine autoreceptors¹⁴⁻¹⁶, presynaptic α_2 and opiate receptors^{15,17} or serotonin receptors¹³, as a concentration of $1 \mu\text{M}$ of various antagonists for these receptors, (S-sulpiride, fluphenazine, phentolamine, naloxone, methysergide) did not significantly antagonize the inhibitory effect of melatonin (Fig. 2).

It is not yet known whether the rabbit retina contains melatonin, but the potent effect of this hormone in inhibiting the calcium-dependent release of ^3H -dopamine may have important physiological consequences in the modulation of dopaminergic activity in retina. For example it has been shown that exposure of dark-adapted animals to light significantly increases the activity of tyrosine hydroxylase^{9,12} and the rates of dopamine release¹⁰, synthesis¹¹ and turnover^{11,12} in rat, rabbit and cat retina. The mechanism(s) involved in the light-induced increase in activity of retinal dopamine neurones is not known¹¹. The results of this publication lead to the suggestion that, if melatonin is found in the retina of the rabbit as reported in other species²⁻⁸, light might modulate the activity of the dopamine-containing amacrine cells of the retina indirectly, through melatonin, since its synthesis via N -acetyltransferase is regulated by environmental light^{3,8}. For example in darkness, depolarized photoreceptors produce and secrete melatonin²⁰ while exposure to light, by rapidly inactivating N -acetyltransferase^{3,8}, leads to a decrease both in melatonin synthesis and secretion from the hyperpolarized photoreceptors²⁰. It might be suggested that during the dark-period the elevated concentration of melatonin in the retina^{3,8}, exerts an inhibitory action on the activity of retinal dopamine neurones. On the contrary the low levels of melatonin during the light period might disinhibit the dopamine-containing amacrine cells of the retina, leading to an increase in tyrosine hydroxylase activity and dopamine release, synthesis and turnover⁹⁻¹².

The light-dependent production of melatonin may modulate not only eye pigmentation²⁰ and photoreceptor metabolism²¹, but also the activity of dopamine-containing amacrine cells in retina in physiological conditions. Finally, if the dopamine

neurones are involved in adaptation of the retina to light, as suggested by Ehinger²², it might be speculated that melatonin by inhibiting and disinhibiting the dopamine amacrine cells in dark and light respectively, might be a physiological mediator of light adaptation.

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Patch- and voltage-clamp analysis of cyclic AMP-stimulated inward current underlying neurone bursting

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The second messenger cyclic AMP has been variously reported to affect the electrical activity of different neurones by decreasing outward potassium current^{1–3}, increasing outward current⁴ and increasing inward current^{5–8}. The recently developed patch clamp method of recording single ionic channels⁹ allows direct measurement of the action of cyclic AMP on membrane conductances. Using the patch clamp, the closure of potassium channels by cyclic AMP has previously been documented on the single channel level¹⁰. We report here that in a bursting molluscan neurone, intracellular iontophoresis of cyclic AMP under voltage clamp elicits an inward current of maximal amplitude in the pacemaker voltage region. Patch-clamp analysis reveals inward channels whose opening frequency is augmented by cyclic AMP stimulation and whose activity accompanies burst episodes. Channel opening frequency is significantly increased by depolarization of the whole soma, but not by focal depolarization of the patch; this may reflect the action of another second messenger that acts in concert with cyclic AMP to confer voltage sensitivity.

The bursting cells that we studied were the paired ventral white cells (VWCs) of the marine mollusc *Pleurobranchia*¹¹. Buccal ganglia were dissected from *Pleurobranchia* (20–100 g) and pinned to a layer of Sylgard on the bottom of the recording chamber. Saline composition was (in mM): 420 NaCl, 25 MgCl₂, 25 MgSO₄, 10 KCl, 10 CaCl₂ and 5 MOPS, adjusted to pH 7.5 at 13 °C. For voltage clamping, VWCs were axotomized and stabilized against insect pins¹². A single-barrelled voltage electrode and a double-barrelled current electrode were inserted

into the VWC. One barrel of the current electrode was filled with 0.2 M cyclic AMP adjusted to pH 7.4 with KOH. Current was passed between the two barrels to iontophorese cyclic AMP. Permanent records were made on a Gould 2400 chart recorder.

To clean the membrane surface for patch clamping, the cells were bathed in 0.25% trypsin for 90 min. All VWCs survived trypsin treatment with normal resting potentials (–50 to –60 mV) and overshooting spikes. The somata were voltage clamped with a single electrode voltage clamp (Dagan 8100) and single channels were recorded using a Dagan 8900 patch-clamp apparatus⁹. Patch pipettes were filled with saline having the same composition as the bath saline. Seal resistances were 5 to 20 GΩ. The signal was recorded on a digital storage oscilloscope and on an FM tape recorder for storage. The tape recorder was played back at a slower speed onto the chart recorder for single channel frequency analysis. After an inward channel was isolated, and baseline activity was established, the bath was perfused with a membrane soluble, cyclic AMP analogue *p*-chlorophenylthio adenosine 3'5' monophosphate (CPT-cAMP; ICN Biochemicals)¹³ at 10^{–4} M.

Under voltage clamp, cyclic AMP iontophoresis elicited an inward current. Peak current responses of over 10 nA could be elicited, but injection current was purposely kept low to avoid jamming of the current electrode. Injection of the non-cyclic 5' AMP was without noticeable effect. Figure 1A shows a typical current response to intracellular cyclic AMP injection. The amplitude of the current response to injected cyclic AMP varied with the holding potential, as is shown in Fig. 1A and B. Cyclic AMP induced inward current could be recorded at holding potentials as low as –90 mV. The potassium equilibrium potential has been estimated by us and other researchers^{10,14} to lie between –65 to –75 mV, suggesting potassium is not a major charge carrier of this current. The voltage region (–20 to –30 mV) that supports the largest cyclic AMP current coincides with the negative slope region in *I*–*V* curves for the VWC and other bursting neurones that is indicative of slow inward current^{12,15–17}. This correlation of effective voltage ranges suggests the cyclic AMP current contributes to slow inward current.

Of all the different types of inward and outward current channels recorded in the patch clamp experiments, only inward channels were cyclic AMP-responsive while the soma was voltage clamped. Inward current channels were recorded in ~35% of all successful patches. Inward channel open times ranged from 1 to 20 ms, and the frequency of occurrence ranged from 0 to 10 openings per s. Conductance through the inward channels was approximately ohmic, with conductances around 20 pS at 13 °C. Voltage clamping of the patch pipette revealed a reversal potential for these channels of +40 to +50 mV. In four experiments where the cell was voltage clamped to –50 mV, and in two at –60 mV, CPT-cAMP increased the average opening frequency from 1.45 ± 0.35 to 3.1 ± 0.48 openings per second. The opening frequency was sampled for a minimum of 3 min before and after addition of CPT-cAMP. In two cells, opening frequency was sampled for up to 15 min, but the frequency did not differ significantly from a 3-min sample. In a typical experiment, bath addition of CPT-cAMP significantly increased the opening frequency by 140% from 1.25 ± 0.19 to 2.98 ± 0.32 openings per second. Mean open time of the channel in CPT-cAMP (8.00 ± 0.87 ms) did not change significantly from baseline (7.21 ± 0.87 ms).

In the presence of CPT-cAMP, channel opening frequency increased before changes in whole cell electrical activity, and while the VWCs were voltage clamped (Fig. 2), indicating the frequency was modulated by CPT-cAMP, and not ionic changes induced by action potentials. Single channel opening frequency increased 10–20 min after bath addition of the cyclic AMP analogue¹⁸, and was temporally correlated with a more negative holding current (increased inward current) under voltage clamp. In unclamped cells, spontaneous burst episodes were initiated within 1–5 min after clear increases in channel opening frequency were detected. During a CPT-cAMP initiated burst, action potentials progressively broadened concurrent with an

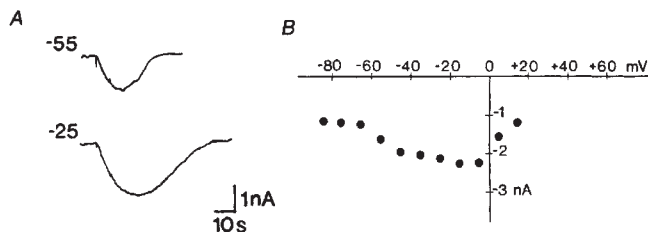


Fig. 1 Intracellular iontophoresis of cyclic AMP elicits inward current. **A**, Current response to 10-s injection of 0.2 M cyclic AMP at holding potentials of -55 and -25 mV. The VWC was axotomized and two electrode voltage clamped. Note increased amplitude at -25 mV. Response amplitude was relatively constant at a given voltage, while response decay rate varied during the experiments. Stimulus artefacts mark onset and offset of injection current, filtered at 5 Hz. **B**, Peak amplitude current response to equivalent five second injections of 0.2 M cyclic AMP at various holding potentials. The cell was allowed to reach a stable holding current at each voltage before cyclic AMP was iontophoresed. A stable holding current could not be obtained at voltages more positive than +15 mV.

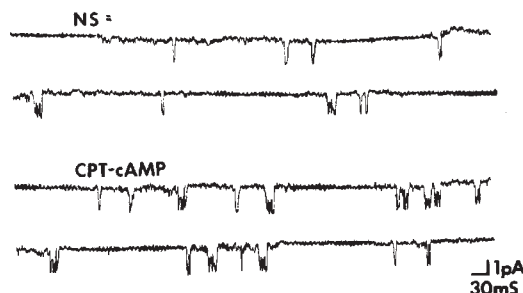


Fig. 2 Effects of CPT-cAMP on single channel activity. CPT-cAMP increased single channel activity while the cell is voltage clamped at -50 mV. Upper two traces are continuous baseline recordings of single channel activity in normal saline (NS). Lower two traces are continuous recordings 10 min after bath addition of 10^{-4} M CPT-cAMP.

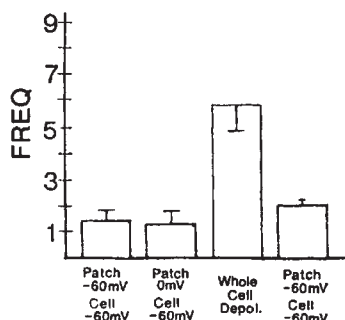


Fig. 3 Channel opening frequency elicited by changes in whole cell or patch pipette potential. The four measurements were taken from the following four conditions. (1) Initially, the cell was clamped at -60 mV, and the pipette to 0 mV, relative to ground. Thus, the patch potential (the voltage across the channel) was -60 mV. (2) While the cell was held at -60 mV, the pipette potential was brought to -60 mV relative to ground, so the effective voltage across the channel was 0 mV. Opening frequency was not significantly changed at this or any other trans-channel potential up to +20 mV. (3) The whole cell was depolarized to approximately -10 mV and allowed to fire action potentials for 15 s. Channel frequency was averaged for the entire 15-s depolarization and was found to increase markedly. (4) Upon re-hyperpolarization to condition (1), channel frequency returned to baseline level within 5 s. Thus, the channel is not directly affected by voltage (for example, when pipette potential is changed), but is activated by changes due to whole-cell depolarization. Error bars indicate standard error of the mean; frequency is number of channel openings per s.

additional increase in channel opening frequency, suggesting that the increased inward channel activity might also contribute to spike broadening. Channel and whole cell electrical activity returned to baseline levels upon CPT-cAMP washout. A previous report has demonstrated closing of outward potassium channels by cyclic AMP in *Aplysia*¹⁰, but we observed no such effect in our preparation.

The single current channels stimulated by the cyclic AMP analogue are probably the class which conducts cyclic AMP-induced slow inward current in the VWCs. These single channels were the only cyclic AMP-sensitive type encountered and were found to be especially active during burst episodes known to be supported by cyclic AMP-activated slow inward current.

Depolarizing the patch up to +20 mV for as long as 10 s had no discernible effect on either opening frequency or open time. However, depolarizations of the whole cell increased opening frequency up to fourfold (see Fig. 3 for details). Thus, the voltage dependence of the slow inward current (Fig. 1B) is reflected on the single channel level by augmented opening frequency during depolarization (Fig. 3). However, the influence of transmembrane voltage on single channel opening is not mediated through a direct effect, since focal depolarization of the patch alone has no effect on opening frequency (Fig. 3). Voltage-induced increases in opening frequency were found to occur only when the whole cell was depolarized (Fig. 3). The difference in the effect of focal versus whole cell depolarizations suggests that stimulation of channel opening could be dependent upon the depolarization-induced accumulation of some factor internally in sufficient quantity to affect the channel. Such a factor would in effect act as another second messenger in synergy with cyclic AMP, and would impart the observed voltage-dependence of the current. The activation of this current by depolarization is reminiscent of a previously described, nonspecific inward cation current which is specifically activated by intracellular calcium ions^{19,20}.

The VWCs of *Pleurobranchaea* are critical loci in the neural network underlying feeding behaviour^{21,22}. Their marked qualities of intrinsic functional plasticity are under neuromodulatory control and are directly reflected in the expression of behaviour²¹. The documentation of cyclic AMP-stimulated increases in opening frequency of single channels identifies one type of fundamental determinant of functional and behavioural plasticity in this system at the membrane level. Similar channels modulated by ligand-receptor-adenylate cyclase interactions have been identified in other systems²³, suggesting that the channels of the VWC belong to a phylogenetically widespread class of inward current channels regulated by phosphorylation.

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Earliest sensory nerve fibres are guided to peripheral targets by attractants other than nerve growth factor

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Recent studies have shown that developing nerve fibres grow directly to their targets¹⁻⁴ and are guided by specific cues^{5,6}, but the nature of these cues and the mechanism of guidance remain unknown^{7,8}. The growth of sympathetic axons towards an artificial source of nerve growth factor (NGF) *in vivo*⁹ and of sensory neurites up a concentration gradient of NGF *in vitro*^{10,11} has supported the hypothesis that NGF, produced by target tissues, acts as a chemotactic attractant for these nerve fibres during development¹². Both these studies and those of the influence of NGF^{13,14} or target tissues¹⁵⁻¹⁹ on neurite growth *in vitro* were conducted late in development when, following target encounter, the neurones had become dependent on NGF or target for survival. Here we have co-cultured embryonic mouse sensory neurones and their peripheral target tissue at a stage preceding their contact *in vivo*. Neurites grew directly and exclusively towards their own target but not to regionally inappropriate peripheral tissue. Antiserum to isogenic NGF did not reduce this outgrowth but did reduce undirected neurite outgrowth which occurred in co-cultures of older neurones with denervated target tissue. These results demonstrate that agents other than NGF guide neurites of NGF-responsive neurones in development.

In normal development of the mouse²⁰ the trigeminal ganglion appears during embryonic day 9 (E9, day of vaginal plug is E0)

and by E10 both the ganglion and the whisker field, a part of its presumptive innervation territory, become discernible (Fig. 1). At E10 about 1,200 fibres extend towards the maxillary process, roughly 5% of the 26,000 peak at E13. Fibres first reach the whisker field epithelium early in E11 and invade the differentiating whisker follicles during E12.

In our procedure (Fig. 1), ganglion explants from E10, E11 and E12 mouse embryos were each co-cultured in collagen gels with two tissue explants of the same developmental age and the same approximate volume; the whisker field (comprising the superficial 100 μm of the rostral half of the maxillary process) on one side and the distal 200 μm of the forelimb bud on the other. These tissues were considered to be broadly homotypic in that both were composed (at E10-E11) of bilaminar ectodermal epithelia and subjacent undifferentiated mesenchyme and both develop structures which receive cutaneous sensory innervation. The limb bud thus provided an alternative, inappropriate target and also acted as control for possible nonspecific contact guidance effects mediated by alignment of fibrils in the collagen gel matrix, which can result from mechanical stresses²¹ and cell traction²². The cultures were incubated for 48 h, examined by phase contrast microscopy and the number, length and orientation of neurites emerging from each quadrant (Fig. 1) of the ganglion recorded.

There was a clear prevalence of exclusively target-directed outgrowth (Figs 2, 3) from E10 and E11 ganglia towards maxillary process tissue. In no cultures of these ages were neurites extended towards the control tissue (Fig. 3). With E12 cultures there was a lower incidence of exclusively target-directed outgrowth, a higher incidence of lateral outgrowth and, for the first time, there was outgrowth towards the controls. There was no neurite outgrowth from ganglia cultured in isolation at any stage ($n = 18$).

Anti-NGF (Fig. 2) had no effect on the outgrowth from E10 ganglia. With E11 ganglia it resulted in both a small but significant reduction in the length of target-directed neurites (control medium $\bar{x} = 380 \pm 20 \mu\text{m}$, anti-NGF $\bar{x} = 280 \pm 30 \mu\text{m}$, $P < 0.01$ Student's *t*-test, Fig. 1) and a small reduction in the overall number of ganglia exhibiting outgrowth. With E12 ganglia however, anti-NGF effected a large reduction in the number of explants exhibiting outgrowth and greatly reduced the lengths

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Fig. 1 Camera lucida drawing of a whole mount silver-stained 10-day (E10) CD1 mouse embryo with schematic representation of procedure in which explants of the developing trigeminal ganglion, whisker field target tissue and limb bud control tissue were co-cultured in collagen gels (to scale). In one set of experiments (left) the three explants, each oriented randomly, were aligned with the trigeminal ganglion (G) between the whisker field and limb bud explants and spaced by $0.5 \pm 0.15 \text{ mm}$, approximately the distance apart of the ganglion and whisker field in E11 embryos. In another set of experiments (right) the control tissue was omitted and a second ganglion explant (G2) was aligned in tandem with the first at a distance not exceeding 1 mm from the whisker field target. Identical sets of experiments were performed with E11 and in some cases also E12 embryos. In all experiments equal-sized explants were submerged in 0.5 ml collagen medium, freshly made with bovine dermal collagen solution (Vitrogen, Flow Labs) 16 parts, 10 $\times$ minimal essential medium (Gibco) 2 parts, 0.08 M NaHCO_3 and 0.2 M NaOH (v/v), in 16-mm diameter multi-well plates (Linbro). Following gelation at 37 $^\circ\text{C}$ for 15 min, 0.1 ml heat-inactivated fetal calf serum (Sera Lab) and 0.4 ml medium 199 (Gibco) were pipetted into each well. Parallel wells each received 2 μl of a solution (2 mg ml^{-1} total serum protein) of sheep anti-mouse submandibular gland 2.5S NGF antiserum, an amount which completely blocked the activity of 2 BU ml^{-1} 2.5S NGF in the standard biological assay. No antibiotics were added to the cultures which were incubated at 37 $^\circ\text{C}$ in 2% CO_2 /air at high humidity and examined at 24, 48 and 72 h by phase contrast microscopy. At 48 h the number, length and orientation of neurites emerging from each quadrant of the ganglion were measured (T, target quadrant; L, lateral quadrants; C, control quadrant or quadrant facing away from target). Neurite length was measured as previously²⁴: the mean distance between the explant boundary and the five growth cones furthest from the boundary in each quadrant gave a score for each quadrant of the explant. The means of the T quadrant scores for all explants within each of the six age/medium categories (Fig. 2) are given in the text (mean $\pm$ s.e.m.) where relevant, as are mean T quadrant and mean C quadrant scores for ganglion explants cultured in tandem. Estimates of neurite number were based on a subjective assessment of neurite density²⁰ within each quadrant made on an ordinal scale from 0-5. Quadrant score medians ($\pm$ interquartile deviations) are given in the text where relevant.

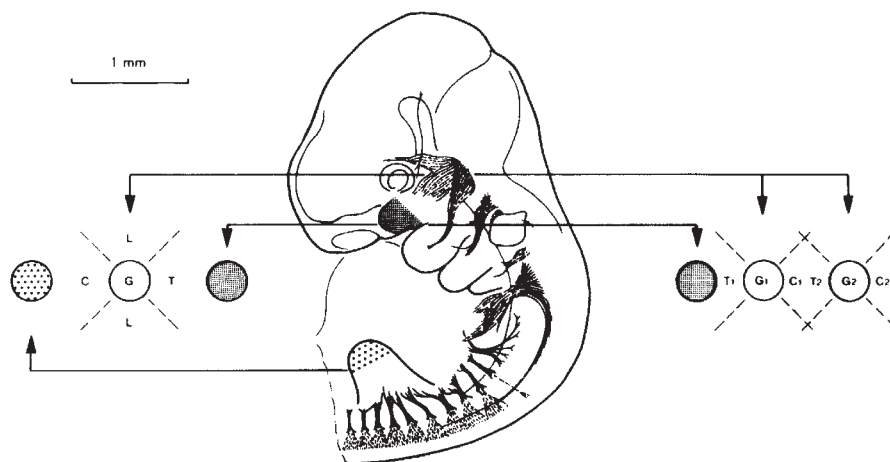
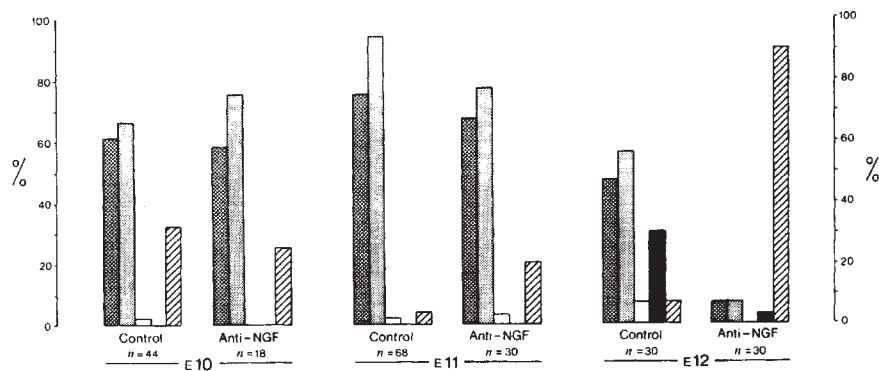


Fig. 2 The pattern of neurite outgrowth in each culture after 48 h was classified into one of the following five categories (bars are numbered from left to right in each group): 'Exclusively target-directed' (bar 1, heavy stipple) includes cultures with neurites only in the T quadrant (for definition see Fig. 1). 'Predominantly target-directed' (bar 2, light stipple) includes the above together with cultures having >75% of neurites in the T quadrant and <25% in L quadrants. Lateral outgrowth (bar 3, unmarked), <75% neurites in T quadrant and >25% in L quadrants. Outgrowth to control tissue (bar 4, black) neurites in C quadrant. No outgrowth (bar 5, hatched). The proportion of cultures falling into each of these categories at each age and in either control medium or medium with anti-NGF are plotted as a percentage of the total number of cultures in each of these six age/medium groups.



of neurites in the remainder (control medium $\bar{x} = 350 \pm 30 \mu\text{m}$, anti-NGF $\bar{x} = 50 \pm 10 \mu\text{m}$, $P < 0.001$).

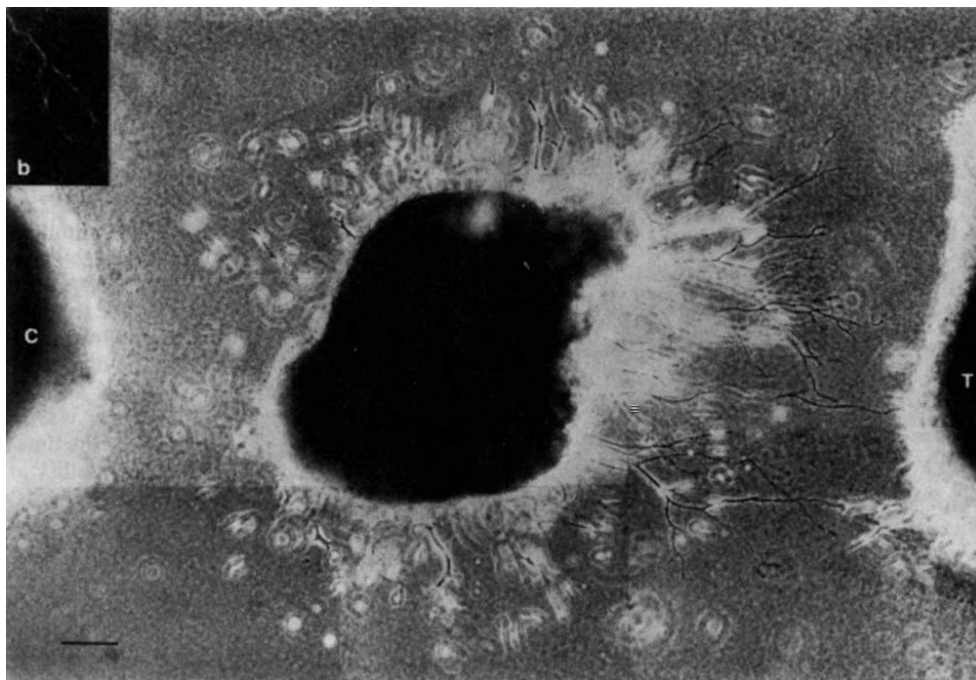
The results demonstrate the existence of an agent, immunochemically unrelated to NGF, which is produced by a peripheral region of the maxillary process and which directs the growth of very early trigeminal neurites. The production, release or response to this agent declines after E11, when nerve fibres reach the periphery during normal development. The agent appears to have some degree of regional specificity as it is not produced by the limb bud which, though also a presumptive cutaneous field, derives sensory innervation from a different source.

An apparent orientation of neurites in explant culture could be due to chemotaxis, that is, dependent on a directional response of the growth cone, or it could result from the activity of a trophic agent enhancing the survival or reinforcing the growth of neurones which happen to be nearest its source²³. An eccentric radial outgrowth pattern with more vigorous extension of neurites from the side of an explant facing a presumed target or source of NGF^{13-15,17} is suggestive of such a trophic effect. The pattern of E10 and E11 neurite outgrowth reported here, which was confined within a quadrant facing an appropriate peripheral target, is more suggestive of chemotaxis. Furthermore, this exclusive pattern of outgrowth persisted

throughout the period of study (up to 72 h), whereas it might be expected of a solely trophic effect that neurites would later appear in the lateral and control facing quadrants as the agent diffused away from the source. Further support for a chemotactic interpretation came from a series of experiments in which cultures were set up at E10 and E11 with two ganglia facing the whisker field in tandem (Fig. 1). In those cases where both ganglia produced neurites (34 out of 55 cultures, at 48 h) outgrowth from the target-facing quadrant of the distal ganglion (quadrant T2, $0.82^{+0.18}_{-0.12}$ mm from the target boundary) was significantly greater than that from the away-facing quadrant of the proximal ganglion (quadrant C1, $0.52^{+0.13}_{-0.10}$ mm from the target boundary): length parameter, T2 $\bar{x} = 170 \pm 17 \mu\text{m}$, C1 $\bar{x} = 36 \pm 8 \mu\text{m}$ ($P < 0.001$, *t*-test); density parameter, T2 median = $1.8^{+0.5}_{-0.6}$, C1 median = $0.4^{+0.5}_{-0.4}$ ($P < 0.001$, Mann-Whitney test). This result indicated that the agent had effectively diffused beyond the proximal ganglion and that visible neurite outgrowth was directed along a presumed concentration gradient towards the target. In accordance with these findings we will refer to this agent as trigeminal neurotropic factor (TNF).

The effect of anti-NGF on E12 cultures revealed a decline in the production or effect of TNF and demonstrated the development of NGF promoted outgrowth. This effect can be interpreted as the production of NGF by normal target and control

Fig. 3 Phase contrast photomontage illustrating the typical pattern of exclusively target (T) directed neurite outgrowth from an E10 trigeminal ganglion in the presence of anti-NGF. No neurites were extended in lateral and control (c) facing quadrants. Scale bar, 0.1 mm. Target-directed neurites consistently extended beyond the non-neuronal cell outgrowth and were confined to the three-dimensional gel. Matrix collagen fibrils, clearly visible in phase contrast, did not align between the explants at the spacing used (0.35–0.65 mm). Neurites were specifically identified in selected cultures at each age by an appropriately controlled standard indirect immunofluorescence method: the inset (b) shows target-directed neurites from an E11 ganglion (cultured in medium containing anti-NGF) reacted with anti-neurofilament monoclonal antibody and stained with rhodamine-conjugated goat anti-mouse immunoglobulin antibody (Nordic).



tissues rather than the maturation of neuronal responsiveness to NGF since both E10 and E11 trigeminal neurones are known to respond to NGF *in vitro* with neurite outgrowth²⁴. There is a considerable body of evidence which suggests that NGF mediates selective survival of sensory and sympathetic neurones during the period of natural neuronal death²⁵⁻²⁸. Our finding that the commencement of NGF production by peripheral tissues coincides with their innervation and just precedes the onset of neuronal death²⁰ strengthens this hypothesis.

Our evidence for the production of a chemotactic agent which guides neurites at the stage during which they normally grow to their targets and the demonstration that NGF is not produced until fibres reach their targets strongly suggests that NGF is not involved in neuronal guidance. Moreover, since the targets of NGF-responsive neurones are ubiquitous, NGF lacks the end-organ specificity required of a natural chemotactic agent⁷. Previous demonstrations of a directional response to exogenous NGF *in vivo*⁹ and *in vitro*^{10,11} may be related to adhesive interactions at the high concentrations of NGF used compared with normal *in vivo* levels^{29,30}. NGF avidly adsorbs onto a large variety of surfaces³¹ and increases the adhesion of growth cones to substrata³², growth cones move preferentially from less to more adhesive substrata³³ and hence possibly along gradients of adhesiveness²³. Recent reports of neurotrophic activity in a variety of tissue extracts and in media conditioned by a variety of different cell types³⁴ indicate that agents other than NGF may promote survival and growth of developing neurones. Several of these agents appear to be substratum associated³⁵⁻³⁷ and, it has been suggested that they are involved in neurite guidance^{38,39}. Whether TNF acts directly or via the substratum remains to be determined and examination of its relationship with other neuronal factors will await the development of specific antibodies.

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Selective growth of natural cytotoxic but not natural killer effector cells in interleukin-3

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Interleukin-3 (IL-3) can promote the proliferation of certain classes of lymphocytes distinct from those that are dependent on interleukin-2 (IL-2) for growth¹⁻⁵. Culture conditions for its production are identical to those required for IL-2 and in both cases the producer cell appears to be Thy 1.2⁺, Lyt1⁺, Lyt2⁻ (refs 1-5). However, unlike the IL-2 responder cells, the cells that proliferate in IL-3 are generally Lyt2⁻ (ref. 1). Here we have measured the natural cytotoxic (NC) activity as well as natural killer (NK) cell activity of the IL-3-dependent cells. Both of these activities are part of a repertoire of spontaneous cytotoxic functions found in mice that might serve in early defence against infectious agents or immunosurveillance against tumours *in vivo*⁶⁻¹². We report a new finding that IL-3 selectively maintains NC cells but not NK cells in culture. This is in contrast to the known requirement for IL-2 in NK cell growth¹³. This provides a means of isolating NC cells from NK cells in the mouse as an initial step in the study of the relative contribution of these two cell types in tumour immunity.

A total of 19 cell cultures, initiated from spleens of BALB/c, NIH Swiss and CBA/N mice, and C57BL/6 with purified IL-3, and maintained in the presence of IL-3 for 3-44 weeks, were tested for cytotoxicity in ⁵¹Cr release assay against two NC targets, WEHI-164 (ref. 6), Meth A (ref. 7) and three NK susceptible targets, YAC-1 (refs 8-10), RLM1 (ref. 9) and RBL-5 (refs 9, 11) (Table 1). The cell cultures showed no detectable activity against the NK target cells in a 4- or 18-h assay. The same IL-3-dependent cells, however, showed significant lysis of WEHI-164. The cytotoxicity against WEHI-164 was a slow process, requiring 18 h of incubation for optimal detection, as has been previously described¹⁴ for NC cells. In expt 4 (Table 1), another NC target, Meth A⁷, was also readily lysed by the factor-dependent cells. In subsequent experiments, all NC activities were measured against WEHI-164. Although these cells exhibited no NK activity, it seemed possible that they might acquire this function on exposure to interferon. Unlike the control fresh CBA/N spleen cells, preincubation of the IL-3-dependent cells with 2,000 units of interferon did not result in lysis of YAC-1 tumour cells, indicating the absence of pre-NK cells that are subject to interferon activation (Table 2). The NC activity of the cell cultures was unaffected by interferon, as is the case with the fresh spleen cells.

Three cell cultures from different strains of mice were followed from the day of initiation in IL-3 for up to 3 weeks (Fig. 1). The high NK activity seen at day 0 was lost within 1 day while NC activity was maintained at approximately the same level throughout the time of testing. The rapid loss of NK activity reflects its high lability at 37 °C, as has been reported earlier⁵. The control spleen cells cultured in the absence of IL-3 also lost NK activity but retained significant NC activity at day 1 (data not shown). These cells, however, did not survive beyond days 3-4 without IL-3 supplement. In fact, the IL-3-dependent cells listed in Table 1 maintained viability only in the presence of IL-3 and could not be weaned off the factor.

The surface markers of the IL-3-dependent cells were compared with those of fresh splenic NC and NK cells from 6-8-

Table 1 NC and NK activities of IL-3-dependent spleen cell cultures

Expt	Strain of IL-3 cultures	Weeks in culture	% Cytotoxicity								
			NC targets			NK targets					
			Meth A 18 h	WEHI-164 4 h	18 h	YAC-1 4 h	18 h	RLM1 4 h	18 h	RBL-5 4 h	18 h
1	BALB/c-1	9		1.8	25.6	-0.2	0.3	0.3	0.8		
	NIH Swiss-1	12		3.4	27.9	-0.4	0.6	-0.2	1.2		
	NIH Swiss-2	12		1.2	15.5	0.5	0.4	0.6	0.9		
	CBA/N-1	8		2.8	35.2	0.4	0.8	0.4	0.6		
	CBA/N-2	8		0.9	18.6	0.3	0.5	0.5	0.4		
2	NIH Swiss-3	15			27.4	0.5				0.2	0.8
	NIH Swiss-4	15			44.1	0.9				0.9	0.7
3	BALB/c-2	14			19.1	0.4				0.3	0.4
	NIH Swiss-5	12			29.5	0.2				0.6	0.5
	CBA/N-3	12			26.8	0.3				0.5	0.2
4	BALB/c-3	20	8.3		8.9	0.8					
	NIH Swiss-6	20	14.8		14.5	-0.2					
	NIH Swiss-7	16	10.3		24.8	-0.5					
	CBA/N-4	16	11.3		18.6	0.4					
	BALB/c-4	3			21.8	0.2					
	NIH Swiss-8	3			15.2	0.6					
	CBA/N-5	3			22.5	0.9					
	BALB/c-3	44			20.9	0.3					
6	NIH Swiss-6	44			19.1	0.1					
	NIH Swiss-7	40			17.8	0.0					
	C57BL/6-1	8			21.4	0.5					
	C57BL/6-2	8			26.8	0.2					

Four BALB/c, eight NIH Swiss, two C57BL/6 and five CBA/N mouse spleen cell cultures were initiated in IL-3 purified by Sephadex G-100 and DEAE column chromatography as previously described¹. Nylon non-adherent spleen cells were seeded at $2-3 \times 10^6$ cells per ml in RPMI 1640 medium containing 10% fetal bovine serum and supplemented with purified IL-3 at a level that yields maximum 20 SDH induction. The cultures were fed twice weekly with purified IL-3 until about day 21 after which they could readily be maintained in 20% WEHI-3 conditioned medium. The WEHI-3 cell line constitutively produces IL-3^{3,5}. The factor-dependent cell cultures were tested simultaneously for NC and NK activities. For the routine measurement of NC activity, 5,000 ⁵¹Cr-labelled WEHI-164 tumour cells⁷ were incubated for 4–18 h at 37 °C with each of the IL-3-dependent cell cultures at effector/target ratios of 200/1 and 50/1, in quadruplicate wells of a microtitre plate. A second NC target, Meth A, was similarly tested in an 18 h ⁵¹Cr-release assay. Measurement of NK activity was identical to the NC assay except that ⁵¹Cr-labelled YAC-1, RLM1 and RBL-5 tumour cells were used¹⁰.

$$\% \text{ Cytotoxicity} = \frac{\text{Experimental c.p.m.} - \text{background c.p.m.}}{\text{Total c.p.m. incorporated}} \times 100$$

The standard errors were usually less than 1% and per cent cytotoxicity of more than 2% were significantly different from the control at $P < 0.05$. In each case, the 50/1 ratio showed a dose-related decrease in cytotoxicity, which was not reported here. Fresh CBA/N spleen cells were included in each experiment and their cytotoxicity against YAC-1 was 24.5–36.8% and that against WEHI-164 was 18–32%, while their cytotoxicity against Meth A was 14.3% in expt 4.

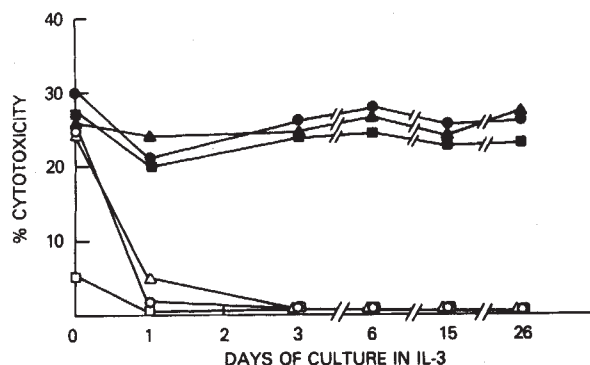


Fig. 1 Kinetics of NC and NK activities in IL-3-dependent spleen cell cultures. Spleen cells were tested before culture in IL-3 and at regular intervals after initiation with the factor. NC activity was measured in BALB/c (●), NIH Swiss (■) and CBA/N (▲) mouse spleen cells, and NK activity was simultaneously measured in the same BALB/c (○), NIH Swiss (□) and CBA/N (△) cells.

week-old CBA/N mice by measurement of residual function after complement-mediated lysis of the appropriate cells. The three representative experiments in Table 3 show that IL-3-dependent cells were very similar to fresh NC cells in showing resistance to treatment with Lyt1, Lyt2, NK1-1, Thy 1.2 and asialo-ganglioside M1 (G_{M1}) but susceptibility to Lyt5 treatment. The IL-3-dependent cells from all the strains tested showed the same phenotype (data not shown). On the other

Table 2 Effect of interferon on cytotoxicity of IL-3-dependent cell cultures

Strain of IL-3 cultures	Preincubation with interferon	% Cytotoxicity	
		NC target WEHI-164	NK target YAC-1
NIH/Swiss-1	–	20.2	0.4
	+	21.0	0.2
NIH/Swiss-2	–	32.4	0.2
	+	31.9	0.6
Control fresh	–	24.8	15.9
CBA/N spleen	+	25.6	34.3

5×10^7 IL-3-dependent cells were incubated with 2,000 units of mouse interferon (Calbiochem-Behring) in 0.2 ml of RPMI 1640 medium containing 5% fetal bovine serum for 1 h at 37 °C. The cells were then diluted and tested for NC activity in an 18-h assay against WEHI-164 and for NK activity in a 4-h assay against YAC-1. As a positive control, fresh CBA spleen cells from 8-week-old mice were similarly treated.

hand, fresh NK cells responded differently to some of these treatments. Splenic NK cells were not inhibited by Lyt1 or Lyt2 but were efficiently stopped by NK1-1, asialo- G_{M1} , and partially inhibited by monoclonal Thy 1.2 and Lyt5. Most of these observations mirror those already reported for fresh NK^{15–21} and NC cells^{7,22–24}. The finding of Lyt5 and H-11 on IL-3-dependent cells, however, represent the first report of recognizable surface antigens on NC cells.

Table 3 Surface markers on IL-3-dependent cells, fresh splenic NC and NK cells

Treatment		% Cytotoxicity		
		WEHI-164 target	Fresh	YAC-1 target
Type	Antibody	IL-3 cells	NC cells	NK cells
Complement-mediated lysis				
Expt 1	None	29.4	36.9	19.8
	Lyt1	28.2	34.6	17.9
	Lyt2	25.1	30.3	18.4
	NK 1.1	26.9	30.8	3.2
	Complement	28.1	32.1	18.8
Expt 2	None	38.9	25.4	24.1
	Thy 1.2	38.1	23.2	14.6
	Asialo G _{M1}	36.8	22.5	2.7
	Complement	37.4	22.1	20.6
	None	24.6	22.4	32.1
Expt 3	Thy 1.2	25.2	27.5	19.4
	Lyt 5.1	10.3	12.2	9.8
	Complement	23.8	25.7	30.4
Fluorescence-activated cell sorting				
		% Positive cells		
		Ig	0	
		Ia	0	
		Mac-1	0	
		Lyt1	0	
		Lyt2	0	
		Lyt5	95	
		H-11	95	

Cells were preincubated with each antiserum for 30 min at room temperature and complement was added for another 45 min at 37 °C before they were washed and tested for the appropriate activities. Lyt1, Lyt2 and Lyt5 were obtained from NEN, while asialo-G_{M1} was obtained from Kasai *et al.*¹⁹ via Dr R. B. Herberman (NCI). NK 1.1 was kindly provided by Dr S. B. Pollack (University of Washington). In expt 1, effector/target ratios shown are 200/1. C57BL/6-1/IL-3-dependent cells and fresh C57BL/6 spleens were used. In expt 2, BALB/c-3/IL-3-dependent cells and fresh CBA/N spleens were used. In expt 3, BALB/c-3/IL-3-dependent cells and fresh CBA/N spleen were used. With Lyt5.1, rabbit anti-mouse immunoglobulin was also added before complement lysis. Fluorescence activated cell sorting was kindly performed by Dr H. C. Morse, (National Institute of Allergy and Infectious Diseases).

Analysis of surface markers, therefore, indicated that the IL-3-dependent cells closely resembled, and may be identical with the fresh NC cells found in the mouse spleen. These cells also resemble fresh NK cells in lacking Lyt1^{16,20}, Lyt2^{16,20} and immunoglobulin^{8,16,20} but containing Lyt5^{17,20} and H-11¹⁶. Although Koo *et al.*¹⁵ were able to detect about 28% of fresh NK cells that reacted with Lyt1, we and others^{16,20} have been unable to confirm this. Other characteristics, however, distinguish the factor-dependent cells from NK cells. The IL-3-dependent cells, like fresh NC cells^{7,22-24}, differ from NK cells by their lack of detectable Thy 1.2¹⁸, NK 1.1^{20,21} and asialo-G_{M1} antigens¹⁹. In addition, they differ from macrophages because they lack adherence properties, Ia and Mac-1 antigens²⁵.

Earlier studies of lymphocyte response to tumours have concentrated on NK cells able to lyse leukaemic cells *in vitro*, and it is now apparent that a wide range of normal and virus-infected fibroblasts as well as solid non-lymphoid tumours are also susceptible to lysis by lymphocytes from non-immune donors²⁶. In addition to this heterogeneity of target cells, there exists a heterogeneity of effector cells, whose contribution to tumour or viral resistance *in vivo* is unknown. Of these effector mechanisms, it has been proposed that NK cells detected by lysis of YAC-1, RLM1 or RBL-5 tumour cells might serve in surveillance against leukaemic cells while NC cells, also termed NK_B

cells by Burton *et al.*¹⁴, reactive against the fibrosarcoma Meth A or WEHI-164 target cells, might be more generally active against solid non-lymphoid tumours¹¹. However, their relationship to each other and their interactions with other host defence mechanisms are unclear. Many of the properties of NK and NC effector functions are the same; for example, pre-existence in high levels in the host without priming, lack of H-2 restriction, ability to lyse more than one tumour cell type, no classical immunological memory, and no well-defined markers of T cells, B cells or macrophages^{14,22}. Other properties distinguish NK cells from NC cells, for example, lack of restriction to strain and age of NC cells^{6,12}, although it now appears that NK activity, absent from the adult spleen, is maintained throughout life in peripheral blood¹⁰. NC cells also show resistance to inactivation by *in vivo* treatment with ⁸⁹Sr, cyclophosphamide, oestrogen and irradiation^{6,22}, all of which can inhibit NK cells²⁷⁻²⁹. In addition, NC cells lack NK 1.1²², NK 1.2^{14,23} and asialo-G_{M1}²³ antigens, which are readily identified on NK cells^{15,19-21,24}.

NK and NC cells are closely related in terms of function but enough differences exist to pose the question of whether they are from separate cell lineages or merely two variants of an effector cell type that mediate most or all spontaneous cytotoxicity. Our observation of the selectivity of IL-3 to maintain proliferation of cells with NC activity, but not NK activity, now provides an important feature that may help to separate the two activities. NK cells have been shown by others to proliferate in IL-2^{13,30}. The different lymphokine requirements for NK and NC cells suggest separate lineage, but can also be interpreted as two maturation pathways or stages of differentiation of a common stem cell. It is possible that NK and NC cells belong to a unique class of lymphocytes that exhibits little or no Lyt1, Lyt2, but expresses Lyt5 and H-11, that, under the influence of IL-2, differentiate into NK cells and, in the presence of IL-3, become NC cells. After differentiation, they may then acquire further markers or properties that distinguish them from each other, for example NK1, asialo-G_{M1}, low levels of Thy 1.2 antigens on NK cells in contrast to drug and radio resistance in NC cells. The lineage of NC cells and their contribution to tumour surveillance and immunity can now be readily approached using IL-3-dependent cells. Recently, Lattime *et al.*³¹ reported that IL-3 augmented NC activity against ⁵¹Cr-labelled WEHI-164 target cells, which is in line with our findings. However, they also suggested that IL-2 can augment NC activity. This latter result is open to question because the IL-2 used was not highly purified and the preparation may contain lymphokines other than IL-2 that can enhance NC activity and are as yet undefined.

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Role of endogenous substance P in stress-induced activation of mesocortical dopamine neurones

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The dopamine (DA) innervation to the forebrain arises from subpopulations of midbrain DA neurones broadly classified as nigrostriatal, mesolimbic and mesocortical¹. Significant differences in the autoregulatory mechanisms and neuronal inputs of these DA pathways may account for their differences in physiological and pharmacological responsiveness². For example, footshock stress can activate rat mesocortical DA cells but does not alter nigrostriatal DA turnover^{3–5}, while also decreasing substance P (SP) concentrations in the midbrain interpeduncular

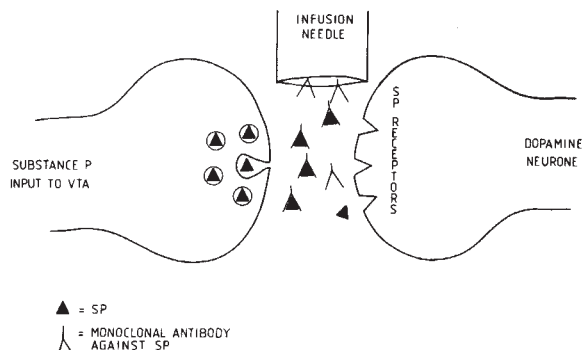
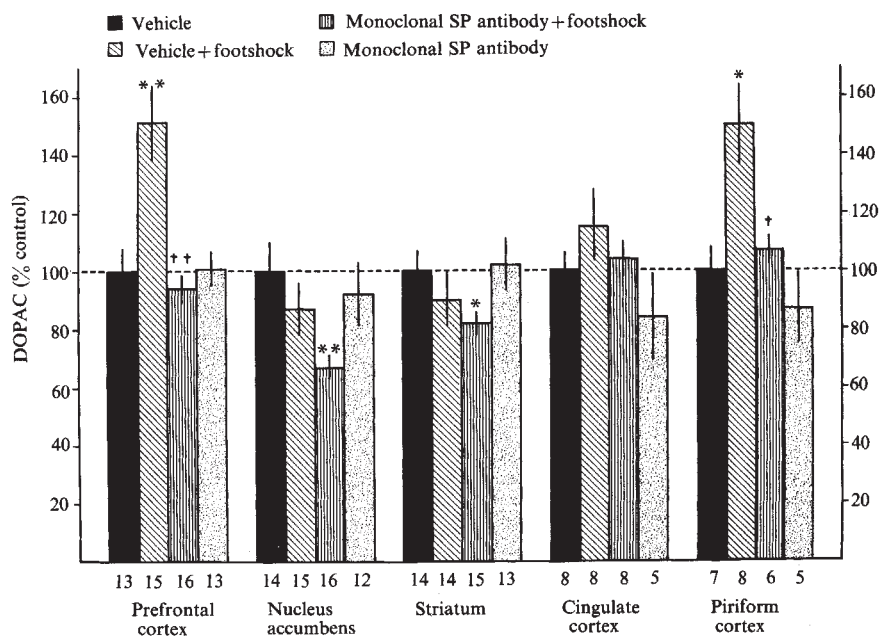


Fig. 1 The effect of infusion of monoclonal antibody against substance P into the ventral tegmental area (VTA) of the midbrain.

nucleus and in the adjacent ventral tegmental area (VTA), but not in the substantia nigra (SN)⁶. This suggested that the activation of the SP input to the VTA⁶ may mediate activation of certain DA systems by footshock stress (Fig. 1); behavioural studies also had suggested an excitatory effect of SP on DA cells in the VTA⁷. SP antagonists now available are neurotoxic⁸ and of questionable efficacy⁹, we therefore used monoclonal antibody against SP. Antibody microinjected into the VTA prevented normal footshock-induced activation of mesocortical DA neurones, suggesting mediation by SP input to the VTA. The *in vivo* application of antibodies may prove valuable in studies of neuropeptides in the central nervous system (CNS).

Adult male rats were exposed to intermittent footshock of very low intensity for 20 min (Fig. 2). A footshock current of 0.2 mA, although significantly less than that commonly used to activate mesocortical DA cells (1.6–2.0 mA)^{3–5}, nevertheless elicited a significant increase in the concentration of the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in the

Fig. 2 The effects of mild intermittent footshock and infusion of monoclonal antibody against substance P on 3,4-dihydroxyphenylacetic acid (DOPAC) levels in various brain regions. Male Sprague-Dawley rats weighing 300–350 g were implanted bilaterally with guide cannulae directed at the VTA¹⁶: (3.5 mm posterior to Bregma, 0.7 mm lateral from the midline, and 8.7 mm below the skull surface¹⁷). After a 7-day recovery period, 2 μ l artificial cerebrospinal fluid or monoclonal antibody (NCI/34 in phosphate-buffered saline) directed against SP¹⁸ (Sera-Lab) were infused bilaterally over a 4-min period as previously described¹⁶. After 1 min, the infusion needles were removed, and the rats were placed individually in a chamber (22×24×21 cm) with floor of stainless steel bars 1.5 cm apart. Shocks of 0.2 mA were intermittently distributed to the bars for 160 ms every 320 ms for 2 s, followed by 8-s periods of no shock for a total of 20 min. A similar shock regimen has previously been shown to increase prefrontal cortical DOPAC without altering brain noradrenergic or serotonergic systems¹⁹. After 20-min exposure to footshock the animals were killed by decapitation and the brains were dissected over ice using previously described procedures for the prefrontal cortex^{12,14,15}, piriform (and anterior entorhinal) cortex^{14,15}, nucleus accumbens¹⁰, striatum¹⁰ and interpeduncular nucleus/ventral tegmental area¹⁰. The DA metabolite DOPAC and dopamine (DA) were extracted on alumina¹², and separated and assayed by high performance liquid chromatography (columns: C₁₈, 5 μ m Spherisorb 50DS2; mobile phase: 0.1 M phosphate buffer containing 0.1% EDTA, 0.25 M octane sulfonate and 15% methanol, pH 3.45–3.6; flow rate: 0.8 ml min⁻¹ coupled to electrochemical detection (Bioanalytical Systems LC-4 amperometric detector, 0.65 V). The bars on the columns indicate the standard error of the mean; the numbers under the columns indicate *n*. Values are expressed as a percentage of control DOPAC levels from either one experiment or two separate experiments prefrontal cortex (53.1±5.4 ng g⁻¹; 47.0±5.2 ng g⁻¹), nucleus accumbens (3,532±426 ng g⁻¹; 2,778±459 ng g⁻¹), striatum (2,360±262 ng g⁻¹; 2,200±188 ng g⁻¹), cingulate cortex (13.0±0.8 ng g⁻¹) and piriform cortex (17.7±1.4 ng g⁻¹). Neither footshock nor antibody infusion significantly altered DA levels in any brain area. **P*<0.02, ***P*<0.005 when compared by an *F*-test with vehicle-treated rats; †*P*<0.02, ††*P*<0.005 when compared with vehicle-treated rats receiving footshock.



prefrontal and piriform cortices (Fig. 2); unlike the more severe shock conditions³⁻⁵, however, there was no change in DOPAC levels in the nucleus accumbens (Fig. 2). As expected, the levels of DOPAC in cingulate cortex and striatum also were unchanged after footshock³⁻⁵ (Fig. 2).

Lisoprawski *et al.*⁶ reported a marked depletion of SP in the interpeduncular nucleus and the VTA of rat brain after exposure to footshock. However, we found that the more mild footshock regime used had no effect on SP levels measured by radioimmunoassay¹⁰ in combined interpeduncular nucleus and VTA (unshocked: 399.7 ± 18.2 pmol g⁻¹ SP, $n = 7$; shocked: 378.8 ± 32.3 pmol g⁻¹ SP, $n = 8$). The footshock-induced changes in DOPAC in the prefrontal and piriform cortices might nevertheless be mediated by changes in SP turnover without any change in SP levels. To test this hypothesis $10 \mu\text{g}$ per side of a rat monoclonal antibody to SP (NC1/34, Sera-Lab) was infused through stereotactically implanted cannulae into the VTA immediately before footshock (Fig. 1). This completely prevented the increase in prefrontal and piriform cortex DOPAC normally elicited (Fig. 2). In control experiments, pre-infusion (Fig. 2) of $10 \mu\text{g}$ per side of a rat monoclonal antibody to mouse IgG (Sera-Lab) did not prevent the footshock-induced increase in prefrontal cortical DOPAC (unshocked 62.9 ± 2.9 ng g⁻¹, $n = 8$; shocked: $85.4 \pm 4.4^*$ ng g⁻¹, $n = 9$, $P^* < 0.02$).

The combination of footshock plus pretreatment with monoclonal antibody against SP produced DOPAC levels in the nucleus accumbens and striatum significantly below those in unshocked controls (Fig. 2). This result is not understood. The lack of effect of monoclonal antibody infusion alone on DOPAC levels (Fig. 2) suggests that the SP input to the VTA may exert little tonic control of the activity of DA cells originating in the VTA. In contrast, Chéramy *et al.*¹¹ found that infusion of SP antiserum into the SN of anaesthetized cats decreased the activity of nigrostriatal DA cells, suggesting that in the cat the SP input to the SN does exert a tonic excitatory influence on those DA cells.

The DA cells with fibres innervating the prefrontal cortex lack autoreceptors, while those DA cells projecting to the piriform cortex possess autoreceptors¹²⁻¹⁵. Yet both these mesocortical DA systems are activated by footshock via a SP-dependent mechanism. This suggests that DA autoreceptors are not involved in response to footshock.

The prevention of footshock-induced changes in prefrontal and piriform cortex DOPAC by prior infusion of monoclonal antibody against SP into the VTA supports the notion that release of SP in the VTA is involved in the activation of mesocortical DA neurones in response to footshock stress.

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A potential donor gene for the *bm1* gene conversion event in the C57BL mouse

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The mammalian major histocompatibility complex (MHC; *H-2* complex in mouse) is a large multigene complex which encodes cell-surface antigens involved in the cellular immune response to foreign antigens¹. Class I polypeptides expressed at the *H-2K* and *H-2D* (Fig. 1) loci of numerous mouse strains exhibit an unusually high degree of genetic polymorphism, which is assumed to be related to their function as primary recognition elements in the immune response. We suggested that this *H-2* polymorphism may arise by gene conversion-like events between non-allelic class I genes. This is supported by our recent comparison of the DNA sequences of the normal *H-2K^b* gene sequence, from the C57BL/10 mouse, and a mutant^{2,3} form of this gene called *H-2K^{bm1}* (ref. 4): the mutant allele differs from the *H-2K^b* gene in seven bases out of a region of 13 bases in exon 3 of the class I gene (which encodes α_2 (C1) the second highly polymorphic protein domain), suggesting that this region of new sequence had been introduced into the *H-2K^b* sequence following unequal pairing of two class I genes in the genome of the C57BL mouse. Schulze *et al.* have obtained similar results⁵. Here we report work identifying a potential donor gene in our library of 26 class I genes cloned from the C57BL/10 mouse.

The region of seven clustered base changes between the *H-2K^b* and the *H-2K^{bm1}* genes is shown in Fig. 2A. As a probe for other class I genes which possess the *bm1*-specific sequence, we synthesized a 15-base oligonucleotide complementary to the *bm1*-specific sequence (Fig. 2A) and labelled it at the 5' end using [γ -³²P]ATP and polynucleotide kinase. The radiolabelled oligonucleotide was then hybridized, in optimal conditions, to *Bam*HI digests of a selection of cosmids containing *H-2* class I genes isolated from a C57BL/10 spleen library (L. Golden, manuscript in preparation). These cosmids contain a total of 26 different class I gene-related sequences as shown by restriction enzyme and hybridization analysis of the cosmid inserts with 5' and 3' class I gene probes. We believe that these genes are most, if not all, of the class I genes in the C57BL/10 genome. The result of the oligonucleotide hybridization to these cosmids is shown in Fig. 2B.

The *bm1* specific oligonucleotide hybridizes strongly to a class I gene in cosmid B1.30 (1.3 kilobase (kb) *Bam* fragment) and to no other cloned class I genes from C57BL/10 mouse DNA. Cosmid BM1-11 is included as a positive control because it was isolated from a *bm1* cosmid library and contains the *H-2K^{bm1}* gene⁴. In the conditions used, the *H-2D^b* gene (cosmid B4.15) does not hybridize to the oligonucleotide probe (data not shown) despite the fact that the DNA sequence matches at 14 out of 15 positions (see ref. 6). Nevertheless, we have seen unreproducible, weak hybridization of the oligonucleotide probe to large *Bam*HI fragments from other cosmids which in some cases (for example, *H26* in Fig. 2B) do not even hybridize to class I gene probes. In one case (cosmid B3.21, not shown), we sequenced through the exon 3 region of the class I gene on such a *Bam*HI fragment and found a sequence with a single mismatch to the *bm1* oligonucleotide probe at amino acid positions 152-156. In contrast, the *bm1* oligonucleotide hybridizes very strongly to the 1.3 kb *Bam*HI fragment in cosmid B1.30 suggesting that there is a perfect match to the *bm1* oligonucleotide probe in

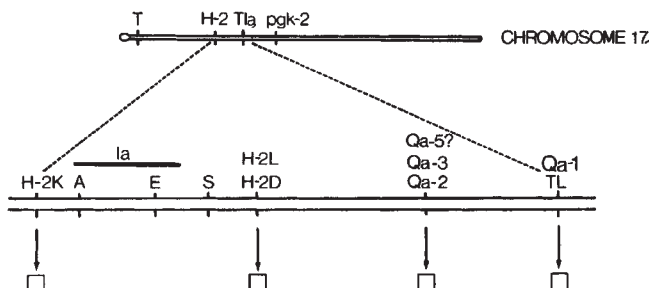


Fig. 1 Genetic map of *H-2* and associated genetic loci on chromosome 17 (top line). Four genetic loci within the *H-2* and *Tla* regions(□) control the expression of class I polypeptides (bottom line).

this class I gene. Cosmid B1.30 contains one complete class I gene and is a member of a large overlapping family of cosmids which together define a region of ~200 kb containing 10 class I genes. The gene (Q10) in cosmid B1.30 maps to one end of this region of DNA which has been shown to map to the *Qa2.3* locus by polymorphic restriction enzyme analysis. Further details of this region of clustered class I genes will be published elsewhere (L. Golden *et al.*; manuscript in preparation).

The 1.3-kb *Bam* fragment hybridizing to the *bm1*-specific oligonucleotide in Fig. 2B was subcloned into the vector pBR327 and restriction maps were deduced as a prelude to DNA sequence analysis. We were able to focus on the region hybridizing to the *bm1*-specific oligonucleotide probe since the 15 bp region includes a *Pst*I site (Fig. 2A). The 1.3-kb *Bam* fragment contains a ~200-bp *Bam*HI-*Pst*I fragment at one end of the subclone which hybridized strongly to 5' class I gene probes containing exon 3. DNA sequence analysis of this region was carried out using the modified Maxam and Gilbert chemical sequencing procedure⁷ (Fig. 3). The DNA sequence obtained is homologous to the DNA sequences found in exon 3 (encodes the α_2 protein domain) of all class I genes which have been sequenced to date^{6,8-10}. In Fig. 4 we present the entire DNA sequence of exon 3 of the *H-2K^b* (see ref. 8) gene and show underneath a comparison of the corresponding homologous sequence obtained from the 1.3-kb *Bam*HI subclone of the Q10 gene. The DNA sequence which encodes amino acids 152-156 is identical to the sequence of the *bm1*-specific oligonucleotide probe. This confirms that the Q10 gene is a potential donor gene for the *H-2K^b* to *H-2K^{bm1}* gene conversion event. In addition, the sequences of this exon 3 region have apparently normal splice acceptor and donor sequences and, moreover, have open reading frames which correlate exactly in size to the *H-2K^b* exon 3 sequence. It is possible, from the DNA sequence, to predict the maximum extent of the sequence transfer if the Q10 gene did take part in the *H-2K^b* to *H-2K^{bm1}* conversion event. Since the DNA sequences of the Q10 and the *H-2K^b* genes first diverges 21 bases to the left of the *bm1*-specific sequence (at amino acid residue 145) and 17 bases to the right (at amino acid residue 162), the maximum extent of gene conversion between these genes is 51 bases.

Since the *bm1* mutation was first detected in a (C57BL/6 × BALB/c)F₁ mouse² it is also possible that the *H-2L^d* gene was the donor gene for this event. In this case, the actual transfer of DNA sequence information from the *H-2L^d* to the *H-2K^b* gene would have had to take place as in interchromosomal event in the zygote as we have stated previously⁴. The maximum extent of gene conversion which could have occurred if the *H-2L^d* gene (bottom line, Fig. 4) had been the donor gene for the *bm1* conversion is 53 nucleotides. It might be argued that the sequence of the Q10 gene in the C57BL/6 mouse differs from that of the C57BL/10 subline. We believe that sequence differences are unlikely to exist for two reasons. First, we have detected only eight base changes (out of a total of ~2,500

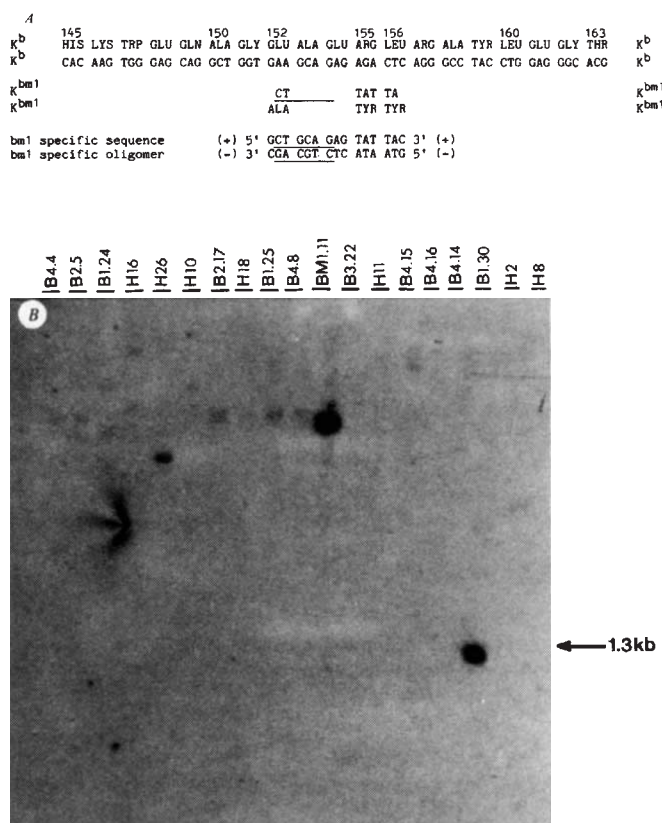
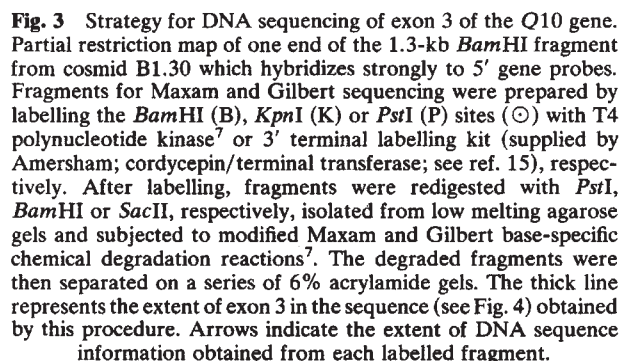


Fig. 2 A, Comparison of DNA and amino acid sequences of the *H-2K^b* (*K^b*) and the *H-2K^{bm1}* (*K^{bm1}*) genes from amino acid 145 to 163. The 15-base *bm1*-specific sequence (+) is shown below the *K^{bm1}* sequence. The complementary 15-base sequence (–) on the bottom line was synthesized manually on a silica gel polymer support by a modification of the published procedure¹². The 5'-OH generated by this procedure was phosphorylated in the presence of [γ^{32} -P]ATP (Amersham) and T4 polynucleotide kinase (NE Biolabs)⁷. *Pst*I sites in the *bm1*-specific sequences are underlined. B, Hybridization of the 15-base radiolabelled oligonucleotide probe in A to *Bam*HI digests of cosmids containing class I genes. 0.6 μ g of each cosmid clone was digested with *Bam*HI, run on a 0.6% agarose gel and transferred to nitrocellulose by the Southern blotting procedure¹³. The filters were prehybridized for 2 h in 6 $\times$ NET (1 $\times$ NET, 0.15 M NaCl, 0.03 M Tris HCl, pH 8.0, 1 mM EDTA), 5 $\times$ Denhardt's, 0.5% Nonidet P-40, 20 μ g ml⁻¹ denatured *coli* DNA at 65 $^{\circ}$ C. Hybridization was performed overnight at 34 $^{\circ}$ –36 $^{\circ}$ C in 6 $\times$ NET, 5 $\times$ Denhardt's, 0.5% Nonidet P-40 and 6 $\times$ 10⁶ c.p.m. of ³²P-labelled *bm1*-15mer. The filters were washed twice in 3 $\times$ SSC, 0.1 SDS, twice in 1 $\times$ SSC, 0.1 SDS, twice in 0.1 $\times$ SSC for 20 min at hybridization or room temperature¹⁴.

nucleotides compared) between the *H-2K^b* (C57BL/10) and *H-2K^{bm1}* (C57BL/6) genes⁸ and seven of these changes are due to the *bm1* mutation itself. This is strikingly high conservation of two *H-2K* genes in a region of otherwise high DNA polymorphism between different mouse strains. Second, we have found, from further sequence analysis of the Q10 gene, that the Q10 gene sequence is almost identical (99.4% conserved) with the sequence of a class I cDNA from a different (SWR/J, *H-2^a* haplotype) mouse¹⁶, suggesting that the cDNA sequence is allelic to the Q10 gene. Thus, if the gene sequence is conserved between two different inbred strains it seems unlikely that there will be any significant variation between the two C57BL sublines.

The discovery that a donor gene for the *bm1* conversion event exists in the C57BL genome provides extra support to our



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Fig. 4 Comparison of DNA and amino acid sequences of the *H-2K^b* gene (top line; from ref. 8), the *Q10* donor gene (centre line) and the *H-2L^d* gene (bottom line; from ref. 9).

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Rearranged *c-mos* locus in a MOPC 21 murine myeloma cell line and its persistence in hybridomas

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Studies of a number of normal and carcinogen-transformed murine cell lines, and a variety of murine tissues, have indicated that, in contrast to several other cellular oncogenes, the oncogene *c-mos* gene is usually transcriptionally silent^{1,2}. The recent report by Rechavi *et al.*³ indicating that in the mouse myeloma XRPC24 originally induced by pristane (2,6,10-14-tetramethylpentadecane) the *c-mos* gene is rearranged and transcriptionally active, and that it can transform murine fibroblasts in a transfection assay, is therefore of considerable interest. Here we show that the *c-mos* locus has undergone a similar rearrangement, and is also transcriptionally active, in the cell line P3-X63-Ag8-653, a derivative of the mouse myeloma MOPC 21 which was induced by mineral oil^{4,5}. This line is widely used for making hybridomas that synthesize monoclonal antibodies^{6,7}. We also demonstrate that the rearranged *c-mos* sequence is maintained in three different hybridomas derived by fusion of this cell line with normal murine spleen lymphocytes, suggesting that it may play a role in the continuous growth and/or constitutive immunoglobulin production by these hybridomas.

Myelomas can be readily induced by intraperitoneal injection of mineral oil or pristane in BALB/c mice⁵. Potter *et al.*⁵ have suggested that only a few genes determine the resistance or susceptibility to myeloma induction by this procedure in various strains of mice. We have examined the arrangement of the *c-mos* sequence in the genome of the myeloma cell line P3-X63-Ag8-653 (abbreviated P3) a derivative of the MOPC 21 myeloma^{4,5}. This line is of particular interest since it is used extensively for the development of hybridomas, because it does not express immunoglobulin chains and fuses very efficiently with antibody-forming cells to form immortal hybrid cell lines that produce homogeneous monoclonal antibodies⁴.

DNA samples obtained from P3 cells and various other cell types were cleaved with specific restriction endonucleases, electrophoresed through a 1% agarose gel, blot hybridized by the Southern procedure⁸ with a probe specific for *c-mos*⁹ and radioautographs of the filters were made. Figure 1 indicates that with DNA from the murine fibroblast cell line NIH 3T3, cleavage with the restriction enzymes *EcoRI*, *HindIII*, *SacI* or *XbaI* gave single bands homologous to the *c-mos* probe, whose sizes were 14.5, 8.7, 6.6 and 2.8 kilobase pairs (kb), respectively. Identical

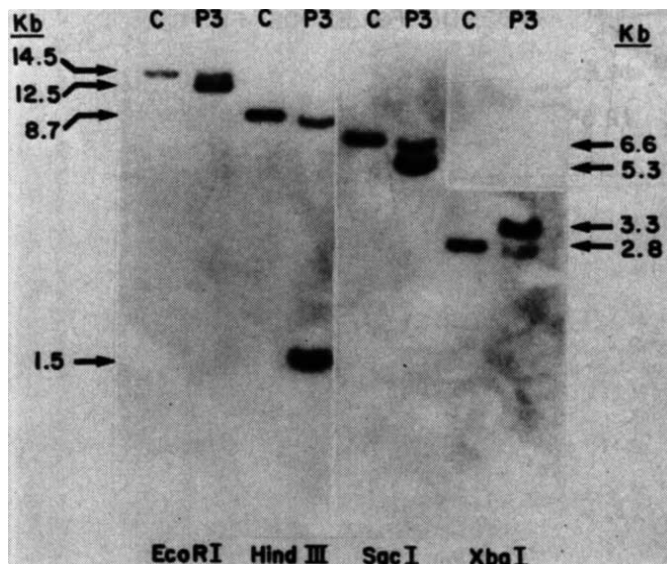


Fig. 1 Southern⁸ blot patterns of the *c-mos* sequences in the genome of NIH 3T3 (C) and murine myeloma cells (P3). Chromosomal DNAs were digested with the restriction endonucleases (New England Biolabs) shown in the figure using the conditions suggested by the manufacturer. The DNAs (10 µg per lane) were electrophoresed through a 1% agarose gel, transferred onto nitrocellulose filter paper and hybridized with a ³²P-labelled¹⁰ probe prepared from an *AvaI-HindIII* *c-mos* specific fragment contained in the plasmid pMS1⁹, kindly provided by Dr George Vande Woude. After hybridization the blots were subjected to autoradiography. The molecular weights of the normal and rearranged *c-mos* alleles are shown by the arrows.

results were obtained with DNA samples from the BALB/c 3T3 fibroblast cell line, two carcinogen-induced transformants of this cell line and normal liver of BALB/c mice (results not shown). In a previous study¹, *SacI*-cleaved DNA samples from a variety of murine cell types also yielded a single band of about 7 kb homologous to a *v-mos* probe. These samples included DNAs from: C3H 10T1/2 fibroblasts, a variety of carcinogen and virus-transformed murine fibroblast cell lines, an AKR (spontaneous) lymphoma, an RF murine lymphoma (methylcholanthrene induced), the F9 murine teratocarcinoma cell line, and several normal murine tissues (spleen, liver, brain, kidney). On the other hand, we now find that with the P3 DNA there appears to be rearrangement of at least one of the *c-mos* alleles, since two rather than one *c-mos* homologous bands were obtained with all four of the restriction enzymes tested. Thus, *EcoRI* digestion yielded 14.5 and 12.5 kb bands, *HindIII* gave 8.7 and 1.5 kb bands, *SacI* gave 6.6 and 5.5 kb bands and *XbaI* gave 3.3 and 2.8 kb bands (Fig. 1).

We also examined three hybridoma cell lines B2, D6 and F8 which were formed by the fusion of P3 cells with normal spleen cells from immunized mice and were selected for the synthesis of monoclonal antibodies to anti Bis Q (B. Erlanger, personal communication). Figure 2 indicates that like the P3 cell line (see Fig. 1), and in contrast to the control sample, all three of the hybridomas displayed two bands with *EcoRI*-cleaved DNA (14.5 and 12.5 kb) and with *SacI*-cleaved DNA (6.6 and 5.3 kb). As with the parental P3 cells, the additional band was in general more intense than the normal band. This was particularly striking with the F8 hybridoma, with both *EcoRI* and *SacI* cleavage (Fig. 2). We have consistently observed this difference in intensity between the normal and additional band in the P3 and hybridoma cell lines, suggesting that the altered *c-mos* allele may have also undergone amplification.

Poly(A)⁺RNA preparations obtained from P3 cells grown under three different conditions (see Fig. 3 legend) gave a single hybridizing band with an apparent size of about 1.2 kbp, when electrophoresed and blot-hybridized to a ³²P-labelled *c-mos*

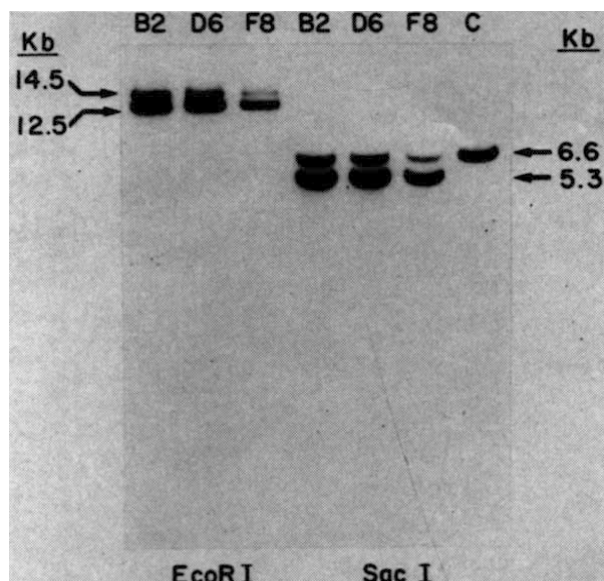


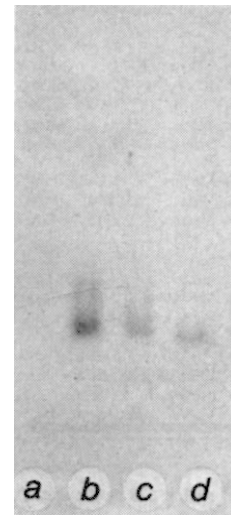
Fig. 2 Southern blots of three different murine hybridoma DNAs (B2, D6, F8) cleaved with the restriction enzymes *EcoRI* or *SacI*, and hybridized with the ^{32}P -labelled *c-mos* probe. Lane C contains *SacI*-digested NIH 3T3 DNA used as a control. The 14.5 and 6.6 kb bands represent the normal allele; the 12.5 and 5.3 bands represent the rearranged allele.

probe (Fig. 3). This finding is similar to that obtained with poly(A)⁺RNA from the XRPC24 myeloma³. As expected, no *c-mos* transcripts could be detected with the poly(A)⁺RNA fraction of the P3 cells or with poly(A)⁺RNA from normal murine fibroblasts (see also refs 5 and 6 for evidence that *c-mos* is not transcribed in a variety of other murine cell types). It should be noted that in P3 cells, and in XRPC24 cells³, the extent of *c-mos* transcription is quite low. Studies in progress utilizing the isoschizomer restriction enzymes *HpaII* and *MspI* suggest that the *c-mos* locus is less methylated in the P3 and hybridoma cell lines than in normal murine cells.

We have also examined by Southern blot analysis a number of human DNA samples, using one or more of the following restriction enzymes, *BamI*, *XbaI* and *EcoRI* and a probe prepared from the cloned human *c-mos* locus¹³. The sources of DNAs included: two human myeloma cell lines and a cell line from a patient with heavy chain disease (kindly provided by J. Buxbaum), an EBV infected lymphoblastoid cell line, five Burkitt lymphoma cell lines, three cutaneous T cell lymphomas, two chronic lymphocytic leukemias, a Bloom's disease fibroblast cell line, peripheral lymphocytes from a patient with dermatomyositis, the K562 and HL60 myelocytic leukemia cell lines, and normal human placenta. None of these revealed evidence for an alteration in the *c-mos* sequence. Thus, although an altered *c-mos* sequence occurs in at least two independently isolated BALB/c mouse myelomas (ref. 3 and the present work) this does not appear to occur, or occurs infrequently, in human myelomas and various human lymphoproliferative disorders.

Rechavi *et al.*³ have cloned the rearranged *c-mos* gene found in the XRPC24 myeloma, and found that it differs from the normal *c-mos* by the presence at the 5' end of a 159-base pair insertion. The sequence of this insert is similar to that of a portion of the LTR sequence of murine intracisternal A particle DNA¹⁴, although the insert is oriented in a direction opposite to that of *c-mos*¹⁵. A comparison of our restriction enzyme analysis of the altered *c-mos* sequence in P3 cell DNA to the published³ restriction map of the rearranged *c-mos* present in XRPC24 DNA suggests that both arose by similar mechanisms. It is clear, however, that the two myelomas were derived independently, using two different chemicals (M. Potter, personal communication). Kuff *et al.*¹⁶ have also detected the insertion of an A particle LTR sequence within a 'k' light chain intron of a murine hybridoma. Since the genome of *Mus*

Fig. 3 Analysis of poly(A)⁺RNA from P3 cells for transcripts homologous to *c-mos*. RNA was extracted from the cells and the poly(A)⁺RNA fraction was isolated on an oligo(dT)-cellulose column¹¹. This RNA was then denatured, gel electrophoresed in 1% agarose, blotted and hybridized to the ^{32}P -labelled *c-mos* probe, using previously described methods¹². Lane a, poly(A)⁺RNA obtained from the oligo(dT)-cellulose column, included as a negative control; lane b, 70 μg poly(A)⁺RNA from subconfluent monolayer cultures of P3 cells; lane c, 65 μg poly(A)⁺RNA from a suspension culture of P3 cells; and lane d, 15 μg of poly(A)⁺RNA from a dense confluent monolayer of P3 cells. Size markers indicate that the band present in lanes b, c and d was 1.2 kb.



musculus contains about 1,000 copies of these A particle sequences¹⁴, it is possible that they may play a rather frequent role in producing gene rearrangements.

Mouse myelomas can display more than one type of alteration in cellular oncogenes. Thus, in addition to the above described changes in *c-mos*, murine myelomas often show reciprocal chromosome translocations involving a region of chromosome 15 that carries *c-myc* and either chromosome 12 or 6 (both of which contain Ig structural genes)¹⁷⁻²¹. Increased expression of *c-myc*, with or without actual rearrangement of its DNA sequence and an altered size of *c-myc* transcripts, have also been observed in various murine myelomas¹⁸⁻²¹. It is likely, therefore, that during the multistep evolution of fully malignant myelomas, and probably other types of tumours, various types of derangements in the structure and function of multiple oncogenes, and other cellular DNA sequences²², can occur. Furthermore, independently isolated tumours, even when obtained within the same experimental system may not show the same constellation of changes. This could in part explain the well-known phenomenon of tumour cell heterogeneity²².

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Rearrangement of the oncogene *c-mos* in mouse myeloma NSI and hybridomas

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The activity and products of cellular oncogenes can be altered by various processes, such as the nearby integration of a retroviral genome, point mutation within the oncogene coding region, gene amplification, and chromosomal translocation (reviewed in ref. 1). Our work has provided an example of oncogene activation by yet a different process; the integration of an endogenous retrovirus-like DNA element (identified as an intracisternal A particle or IAP genome²) within the coding region of the oncogene *c-mos* in a mouse plasmacytoma, XRPC24^{3,4}. The rearranged *c-mos* gene of XRPC24 is actively transcribed and has transforming activity³, suggesting some role for activated *c-mos* in the progression of the XRPC24 tumour. In this report we describe rearrangement of *c-mos* in a second mouse plasmacytoma, NSI, and in two hybridomas. In this case, as in XRPC24, *c-mos* was split by the insertion of a IAP genome. The rearranged *c-mos* genes (*rc-mos*) of NSI and XRPC24 differ in three major aspects: (1) The site of IAP integration in *c-mos* is in codon 30 in NSI but in codon 88 in XRPC24; (2) The orientation of the integrated IAP relative to *c-mos* is 'tail-to-head' in NSI and 'head-to-head' in XRPC24; and (3) transcriptional activity of *rc-mos* in NSI is much lower than in XRPC24. The two latter points suggest a correlation between the orientation of the long terminal repeat (LTR) of IAP relative to *c-mos* and its activity upon IAP integration.

Mouse plasmacytoma NSI (P3-NSI/1-Ag4-1) was derived by Milstein and coworkers from the BALB/c plasmacytoma MOPC21 via the tissue culture adapted cell line P3⁵, and is commonly used as the tumour fusion partner in the generation of hybridomas. DNA from BALB/c, NSI and hybridomas were digested with restriction enzymes and analysed for *c-mos* sequences by Southern blot hybridization. The results shown in Fig. 1 demonstrate that new *c-mos* fragments of 1.1 kilobase pairs (kbp) and 12 kbp were detected in *Hind*III and *Eco*RI digests respectively of NSI DNA (lanes 2, 7). Similar fragments were also apparent in the digests of the two hybridomas (lanes

3, 5 and 8, 10). The *Kpn*I digests of DNA from NSI and the two hybridomas showed a new 4.0 kbp fragment (lanes 12, 14, 15) found neither in BALB/c (lane 16) nor in XRPC24 (lane 13) DNA. Therefore the *c-mos* gene of NSI is rearranged, but differs in structure from *rc-mos* of XRPC24. Hybridoma NSI-103 was generated by fusion of NSI with BALB/c spleen cells, and hybridoma X63-160 by fusion with a related MOPC21 derived myeloma line, P3-X63-Ag8.653⁶. Both hybridomas appear to have acquired and retained the rearranged *c-mos* gene (which we term *rc-mos*^{NSI}) from the respective parental myelomas. The same rearrangement was also found in the DNA of the line P3-X63 which is a tissue culture line of MOPC21. Hence the rearrangement in *c-mos* must have occurred in the original tumor before the drug resistant line NSI was generated.

To determine the structure of the *rc-mos*^{NSI} locus we hybridized Southern blots of various single and double DNA digests from NSI, XRPC24 and BALB/c with three *mos* probes. The first one contains sequences bounded by the *Bgl*I and *Hind*III sites of *v-mos* and detects the 3' 293 codons of *c-mos*⁸. The second probe was the *Xba*I-*Hind*III *v-mos* fragment which detects the 3' 368 codons of *c-mos* (out of 390 codons spanning the entire *c-mos*)⁸ and the third one was an *Alu*I-*Pst*I *c-mos* fragment which spans the first 72 codons of *c-mos* and ~140 bp of 5' flanking sequences. This analysis allowed us to construct the physical map of *rc-mos*^{NSI} as shown in Fig. 2.

Comparison of the *rc-mos* map of NSI to that of germ-line *c-mos* indicates that in NSI *c-mos* was split at the 5' region by the insertion of a ~4.5 kbp long new DNA element. The map of this 4.5 kbp DNA shows a striking similarity to the previously established map of the intracisternal A-particle gene which is integrated in *c-mos* of XRPC24⁴ (Fig. 2 inset). This similarity is only apparent if the orientation of the IAP gene of XRPC24 is inverted. The mapping data clearly indicated that in NSI the site of insertion of this element is upstream from the *Bgl*I site of *c-mos*, which approximately marks the site of IAP integration into *c-mos* in XRPC24³.

To examine the nature of *c-mos* rearrangement in NSI in more detail, we cloned the 12.0 kbp *rc-mos*^{NSI} *Eco*RI fragment in λ vector Charon 4A, and determined the nucleotide sequence of the region between the 5' *Eco*RI site and the *Bgl*I site (see Fig. 2). This sequence is shown in Fig. 3 in alignment with the corresponding *c-mos* sequence⁷, the 5' IAP LTR of *rc-mos*^{X24} (ref. 3), and the 5' LTR of another IAP gene isolated from a BALB/c embryo DNA library. The *rc-mos*^{NSI} sequence is identical with that of *c-mos* 3' to nucleotide 151, but is completely unrelated to it upstream from this position. The new sequence is, however, 93% homologous to that of the complementary strand of the IAP LTR inserted into *c-mos* of XRPC24 (Fig. 3). This shows that also in NSI, *c-mos* rearrangement is due to the insertion of a IAP gene, but that the orientation of IAP

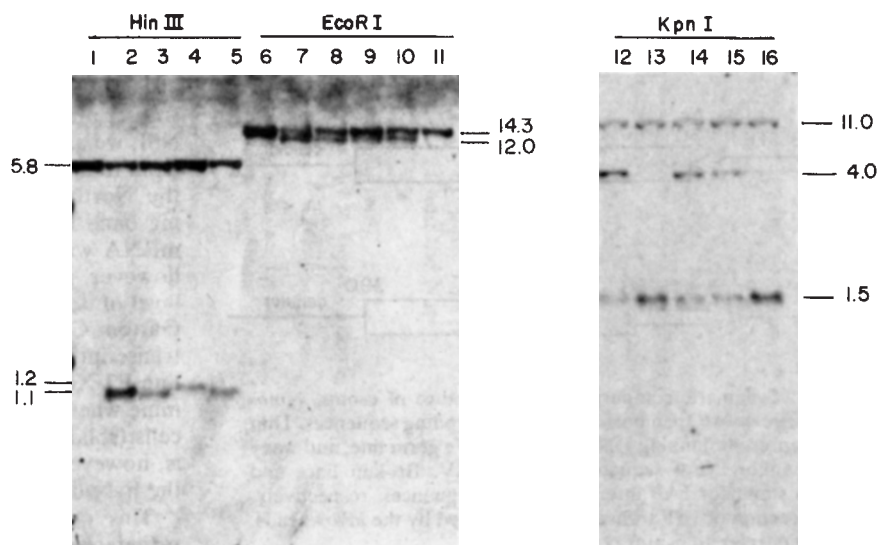


Fig. 1 Southern blot analysis of *mos* sequences in mouse plasmacytoma and hybridoma DNA. High molecular weight DNA (10 μ g) was digested with the restriction enzymes *Hind*III, *Eco*RI or *Kpn*I, size fractionated on 0.7% agarose gel, and transferred to nitrocellulose filters. Hybridization with the radiolabelled *Bgl*I-*Hind*III fragment of *v-mos*⁸ was in 10% dextran sulphate containing 50% formamide. Lanes 1, 11, 16: BALB/c liver DNA; lanes 2, 7, 12: NSI DNA; lanes 3, 8, 14: hybridoma 103 DNA⁶; lanes 4, 9, 13: XRPC24 DNA; lanes 5, 10, 15: hybridoma 160 DNA⁶; lane 6: myeloma MOPC41A DNA.

Fig. 2 Physical maps of the *c-mos* (upper) and *rc-mos*^{NSI} (lower) loci. Coding regions are represented by black bars. The asterisk at the *KpnI* site indicates that the *rc-mos*^{NSI} map was obtained as two unlinked parts, which we joined at this *KpnI* site to give the tentative map of the new DNA piece integrated into *c-mos*. The strategy used to determine the nucleotide sequence of the novel DNA at the 5' end of the cloned region of *rc-mos*^{NSI} is shown by arrows below the map. Inset: comparison of the physical maps of the novel DNAs inserted into *c-mos* of XRPC24 ('X24 IAP') and NSI ('NSI IAP'). Arrowheads indicate the direction of transcription of a normal IAP gene^{21,22}.

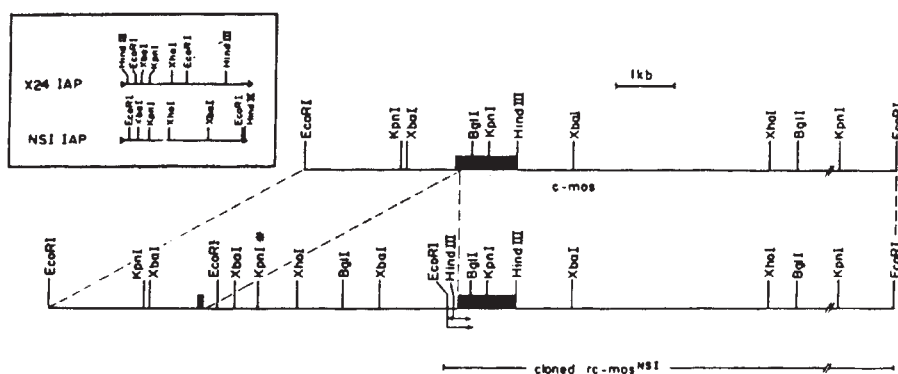


Fig. 3 Nucleotide sequence comparison of *rc-mos*^{NSI} with germ-line *c-mos*⁷ and two IAP LTRs. Sequences were determined on 3' end-labelled fragments according to the strategy outlined in Fig. 4, by the procedure of Maxam and Gilbert^{23,24}. The IAP sequence from *rc-mos*^{X24} (LTR X24 and IAP X24) shown here, is complementary to the sequence that we originally reported^{2,3}. The nucleotide sequence of the corresponding regions in a IAP clone (IAP3.2), which we isolated from a BALB/c germ-line DNA library, is given for comparison. Dashes indicate identity to the *rc-mos*^{NSI} sequence, and open spaces stand for deletions. Numbers refer to nucleotide positions in the *rc-mos*^{NSI} sequence, starting at the *EcoRI* site. Amino acid residues of the *c-mos* gene product are given in the one letter code above the *c-mos* sequence. A putative polyadenylation signal at nucleotides 78–83, and the AACA tetranucleotide marking the end of the IAP LTR are underlined. The position in *c-mos* corresponding to the *mos*/helper virus junction in Mo-MuSV strain 124^{8,9} is marked by a triangle. Nucleotide differences between IAP X24 and IAP 3.2 in the body of IAP are indicated by asterisks. Note that none of the LTRs contain a 'TATA'-like sequence at the position (centred around the *EcoRI* site of the NSI sequence) which Kuff *et al.*²⁰ suggested as the site of a Goldberg-Hogness box on the basis of the LTR sequence of their IAP clone, λ MLA14.

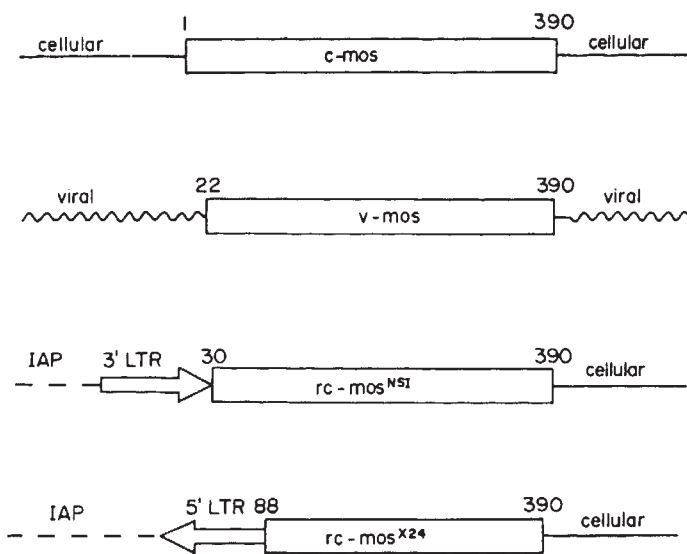
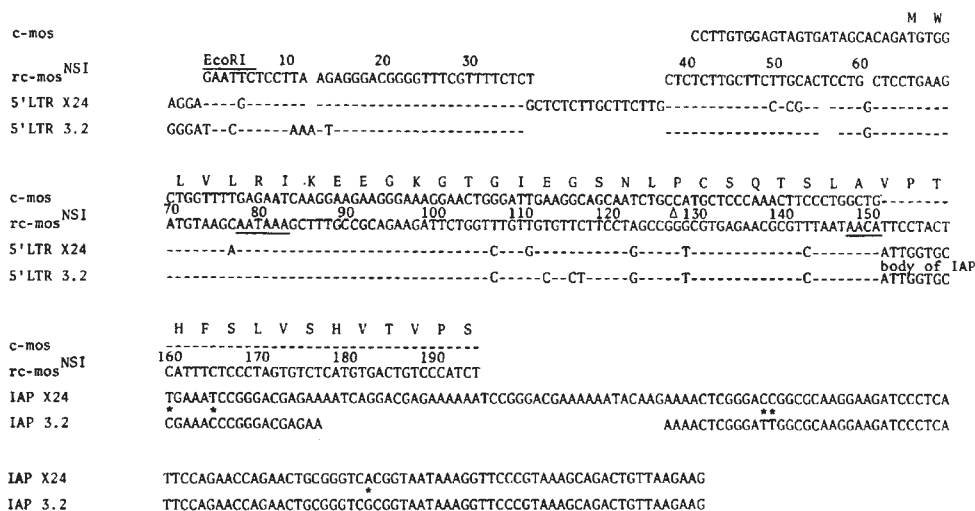


Fig. 4 Schematic comparison of the structure of *c-mos*, *v-mos* and two *rc-mos*. Open boxes delineate the structure of *c-mos*, *v-mos* and two *rc-mos*. Thin lines represent flanking DNA in the BALB/c germ line, and wavy lines flanking viral sequences in Mo-MuSV. Broken lines and arrows stand for IAP internal and LTR sequences, respectively. The direction of IAP transcription is indicated by the arrowheads. Numbers refer to *c-mos* codons.

relative to *c-mos* is opposite in the two cases: in NSI the IAP sequences are in the same transcriptional orientation as *c-mos* (tail-to-head) whereas in *rc-mos*^{X24} the IAP gene is joined to *c-mos* head-to-head. The site of insertion also differs between NSI and XRPC24. The sequence shows that the IAP LTR joined *c-mos* at codon 30 in NSI, which is 8 codons downstream from the *v-mos*/helper virus junction in the genome of strain 124 of Mo-MuSV^{8,9}, and 57 codons upstream from the IAP integration site of *rc-mos*^{X24} (ref. 3). Figure 4 schematically shows the differences between four naturally occurring versions of the *mos* oncogene: *c-mos*, *v-mos*, *rc-mos*^{NSI} and *rc-mos*^{X24}.

To investigate the effect of the DNA rearrangement on the transcription of *mos* in the two myeloma lines XRPC24 and NSI, we analysed the poly(A)⁺ RNA from these tumours, and from tumours of the two hybridomas, for *mos* transcripts using the 'Northern' technique. The results showed a strong hybridizing band of 1.2 kb in XRPC24 RNA whereas no hybridizing mRNA was detected in the NSI and hybridomas RNA. It is, however, possible that the *mos* mRNA in NSI was below the level of detection in our experiment. In a similar type of study Gattoni-Celli *et al.*¹⁰ have detected a small amount of *mos* transcript in mRNA extracted from tissue culture cells of the line P3-X63-Ag8.653. Further studies are necessary to determine whether the different results are due to the source of the cells (solid tumour versus tissue culture) or to other factors. It is, however, clear that the level of *mos* transcript in NSI and the hybridomas is much lower than in XRPC24.

This quantitative difference may be correlated with the orientation of the LTR with respect to *c-mos* in the two

plasmacytomas (see Fig. 4). Such a correlation would emphasize the similarities between IAP genetic entities and the yeast Tyl transposable elements, since activation of neighbouring yeast genes upon Tyl insertion occurs only when the transposon is inserted head-to-head^{11,12}. Alternatively the difference in transcriptional activity of *rc-mos* in NSI and XRPC24 might be due to structural differences between the 5' LTR and the 3' LTR. Unlike the LTRs of retroviral proviruses^{13,14} and the direct repeats at the ends of several *Drosophila* transposons¹⁵⁻¹⁷ and yeast^{18,19}, the two LTRs of a single IAP gene are not identical. Multiple differences were found between the two LTRs of the IAP gene that integrated into *c-mos* of XRPC24⁴, and also between the LTRs of another IAP gene, λ MIA14²⁰. This suggests the possibility that the putative IAP promoter of the 5' LTR is less active in the 3' LTR due to differences in nucleotide sequence.

IAP genes are present in numerous copies in the mouse genome. Our work as well as that of others demonstrates their behaviour as movable elements^{3,4,20}. The accumulated data show the different consequences of transposition of these endogenous elements into cellular genes and underline their potential to profoundly alter cellular gene expression. Studies, currently in progress in our laboratory, are designed to investigate the parameters that determine the consequences of IAP integration for transcriptional activity of neighbouring genes.

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Identification of reciprocal translocation sites within the *c-myc* oncogene and immunoglobulin μ locus in a Burkitt lymphoma

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The association between certain human tumours and characteristic chromosomal abnormalities has led to the hypothesis that specific cellular oncogenes¹ may be involved and consequently 'activated' in these genetic recombinations^{2,3}. This hypothesis has found strong support in the recent findings that some cellular homologues of retroviral *onc* genes are located in chromosomal segments which are affected by specific tumour-related abnormalities (see ref. 4 for review). In the case of human undifferentiated B-cell lymphoma (UBL) and mouse plasmacytomas, cytogenetic and chromosomal mapping data have identified characteristic chromosomal recombinations directly involving different immunoglobulin genes and the *c-myc* oncogene (for review see refs 5, 6). In UBLs carrying the t(8:14) translocation it has been shown that the human *c-myc* gene⁷ is located on the region of chromosome 8 (8q24) which is translocated to the immunoglobulin heavy-chain locus (IHC) on chromosome 14⁸⁻¹⁰. Although it is known that the chromosomal breakpoints can be variably located within or outside the *c-myc* locus and within the IHC μ (refs 9, 11) or IHC γ locus¹², the recombination sites have not been exactly identified and mapped in relation to the functional domains of these loci. We report here the identification and characterization of two

reciprocal recombination sites between *c-myc* and IHC μ in a Burkitt lymphoma. Nucleotide sequencing of the cross-over point joining chromosomes 8 and 14 on chromosome 14q—shows that the *onc* gene is interrupted within its first intron and joined to the heavy-chain μ switch region. This recombination predicts that the translocated *onc* gene would code for a rearranged mRNA but a normal *c-myc* polypeptide.

The genome of the ST486 cell line¹³, which was derived from an American, Epstein-Barr virus-negative, Burkitt's type UBL, with a t(8:14) chromosomal translocation, was previously shown to contain a rearranged *c-myc* allele and IHC μ sequences co-migrating in the same restriction enzyme fragment¹¹. To clone this region, which may contain the recombination site(s) between chromosomes 8 and 14, a genomic library, constructed¹⁴ by partial *Mbo*I restriction enzyme digestion of ST486 DNA and ligation/packaging into the phage vector λ -J1, was screened using plasmid pMC415PP DNA as a probe (see Fig. 1a). The plasmid contains the 5' coding exon, 5' intron, the 5' noncoding exon and 5' upstream sequences of the human *c-myc* locus (as illustrated in Fig. 1a). The latter were identified by comparative nucleotide sequence analysis of cDNA and genomic clones (refs 15-17 and M.C.P., R.D.F., T.S.P., in preparation). The 5' noncoding exon is transcribed as part of the 2.3-kilobase (kb) *myc* messenger RNA, but the region contains translational stops in all reading frames¹⁷, suggesting that *myc* protein translation begins at the second exon. Because our previous results¹¹ had indicated that the chromosomal breakpoint was located 5' to the 1.4-kb *Sst*I-*Sst*I restriction fragment containing the second *c-myc* exon, a probe comprising part of this fragment plus a region extending upstream was likely to span the chromosome breakpoint and allow cloning of both recombined *myc* locus on chromosome 14 and residual *myc* flanking sequences on chromosome 8. The positive clones could then be screened with different fragments from *myc* or IHC μ loci to characterize them fully.

Restriction enzyme analysis of six *myc*-containing recombinant bacteriophages allowed us to characterize one clone as containing the normal *c-myc* allele, which originated from the normal chromosome 8 and was not analysed further. The remaining five clones contained rearranged *c-myc* sequences reminiscent of the rearrangements shown in Southern blotting analysis of total chromosomal DNA¹¹. The clones were analysed by hybridization to different IHC μ probes (Fig. 1b) which are

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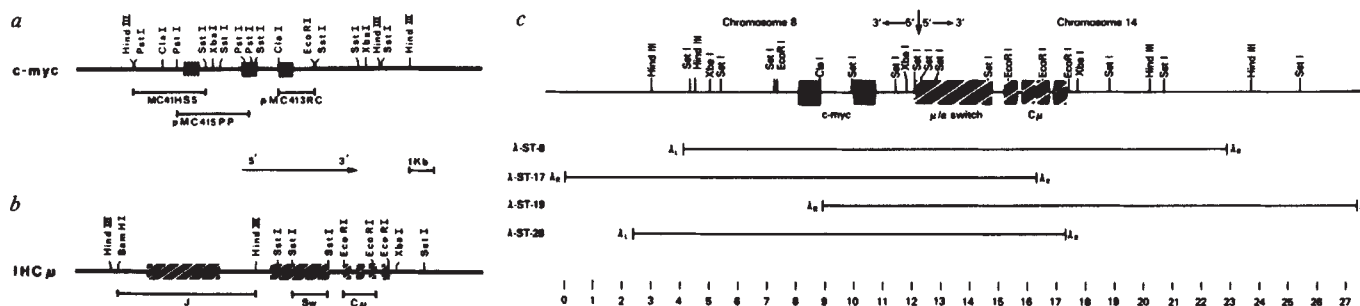


Fig. 1 Genomic organization of normal and recombined *c-myc* and *IHC μ* loci and scheme of probes used in this study. *a*, *c-myc* locus. The restriction enzyme map is derived, with minor corrections, from the published map of the λ -LMC41 clone⁷. Black boxes indicate the second and third exons as characterized by homology with *v-myc* and by nucleotide sequence analysis¹⁵⁻¹⁷. Vertically hatched box represents the first noncoding exon as identified by nucleotide sequence analysis of genomic (λ -LMC41) and cDNA clones^{7,15-17}. The construction of subclones pMC413RC and pMC415PP has been described previously^{12,37}. MC41HS5 probe is a *HindIII*-*SstI* fragment isolated from an *SstI*-*HindIII* digest of plasmid pMC41 (plasmid pMC41 contains the entire *c-myc* gene, located between *EcoRI* (3') and *HindIII* (5') sites). *b*, *IHC μ* locus. Only part of the locus is illustrated, as derived from ref. 18. Boxes indicate functional or coding domains as indicated. Subcloned probes have been previously described¹⁹ and were provided by S. Korsmeyer. *c*, A 27-kb region from chromosome 14q+ of ST486 cell line, containing *c-myc* and *IHC μ* sequences. Symbols are as in *a* and *b* and as indicated below. Recombinant phage clones λ -ST-8, -17, -19 and -28 were isolated from a genomic library of ST486 chromosomal DNA. ST486 DNA was subjected to partial endonucleolytic cleavage by *MboI*, ligated to λ -J1 arms and *in vitro* packaged essentially as described by Maniatis¹⁴. The λ -J1 vector was a gift from J. Mullins. The respective positions of left (λ L) and right (λ R) phage arms is indicated for the different clones. The library has been screened by plaque hybridization¹⁴ using clone pMC415PP as a probe. Hybridization was performed essentially as described in ref. 38. Positive clones were plaque purified, grown in mass and DNA was purified. Different DNA inserts have been mapped and oriented by Southern blotting hybridization³⁹ using the different *myc* and *IHC μ* probes described in *a* and *b*.

representative of different functional areas of this locus^{18,19}. All five clones were shown to contain *IHC μ* sequences and four of them (λ -ST-8, -17, -19 and -28) were found to overlap on a 27-kb genomic region containing *c-myc* and *IHC μ* (switch and *C μ*) sequences (see Fig. 1c). The restriction map directly proved the linkage between *c-myc* and *IHC μ* and confirmed that these two loci were rearranged in a head-to-head (5' to 5') configuration, as 5' sequences had been lost from both loci. The germ-line genomic organization was perfectly conserved in both loci up

to a joining region at the 5' side of *c-myc* and the 5' end of switch where a newly generated ~0.2-kb fragment appeared between two *SstI* sites conserved from the *c-myc* and *IHC μ* loci, respectively. Since chromosomal mapping experiments have shown that in other Burkitt cases the *C μ* domain is retained on chromosome 14 whereas the *c-myc* locus is translocated^{8,20}, we assume that this fragment contains the cross-over point between chromosomes 8 and 14 on chromosome 14q+.

To map and characterize the recombination site further, we performed nucleotide sequencing analysis of the *SstI*-*SstI* restriction fragment by the method of Maxam and Gilbert²¹ (Fig. 2). The resulting sequence was then compared by computer analysis with the sequence of the entire human *c-myc* locus (*HindIII*-*EcoRI* fragment in Fig. 1) (refs 15, 16 and M.C.P., R.D.F., T.S.P., in preparation). This comparison showed that a stretch of 108 nucleotide residues of the *SstI* fragment was identical to a region located within the first *c-myc* intron. In the 5' direction (with respect to the transcription polarity of the unrearranged *c-myc* gene), no further homology with *c-myc* was observed. These remaining nucleotides contained sequences homologous to the *IHC μ* switch with the characteristic pentameric repeated structure of immunoglobulin switch regions (refs 22, 23 and see Fig. 2). From this analysis we assumed that the first *c-myc* intron had been interrupted and had been joined to sequences within the *IHC μ* switch region on chromosome 14. In this case the exact chromosomal breakpoint is located towards the 5' end of the intron, 280 base pairs (bp) downstream from the splicing donor site of the first exon.

Because additional rearrangements could be present within the translocated *c-myc* locus, yet not be detected by restriction mapping, we performed heteroduplex analysis between the normal (λ -LMC41) and rearranged (λ -ST-8) *c-myc* clones. Although minimal rearrangement and mutations could be detected only by sequencing the entire translocated *c-myc* gene, heteroduplex analysis provides reasonably good information about the colinearity of two homologous sequences to a sensitivity of approximately 100 bp. The structure of the heteroduplex molecules (Fig. 3) indicated that no further rearrangements occurred within the *c-myc* gene and approximate measurements confirmed the location of the single chromosomal breakpoint located by restriction enzyme mapping and nucleotide sequencing. A single additional region of nonhomology was detected as a 0.2-kb substitution loop located outside the *c-myc* gene in the 3'-flanking region. This region represents deletion of a fragment downstream from the λ -LMC-41 *c-myc* locus and its

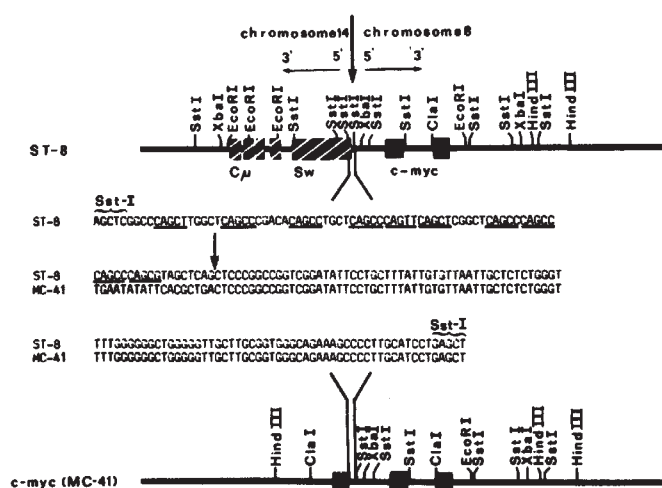


Fig. 2 Nucleotide sequence and localization of chromosomal cross-over point. The newly generated 0.2-kb *SstI*-*SstI* (see text and Fig. 1c) was isolated by *SstI* cleavage of ST-8 and electrophoresis on a 6% acrylamide gel. The fragment was then analysed by the nucleotide sequencing technique of Maxam and Gilbert²¹ and the sequence was then compared by computer analysis with the known nucleotide sequence of the human *c-myc* locus (*HindIII*-*EcoRI* fragment from clone λ -LMC41, ref. 16 and M.C.P., R.D.F., T.S.P., in preparation). A single region of complete homology was identified and aligned to the *SstI*-*SstI* sequence from λ -ST-8. At the site indicated by the vertical arrow the homology with *c-myc* ends and homology with human switch μ region begins. This latter homology is not indicated by alignment of sequences but the pentameric repeated sequences characteristic of switch region^{22,23} are underlined. The localization of the ST-8 and MC41 sequences within their respective clones is indicated by brackets. Symbols are as in Fig. 1.

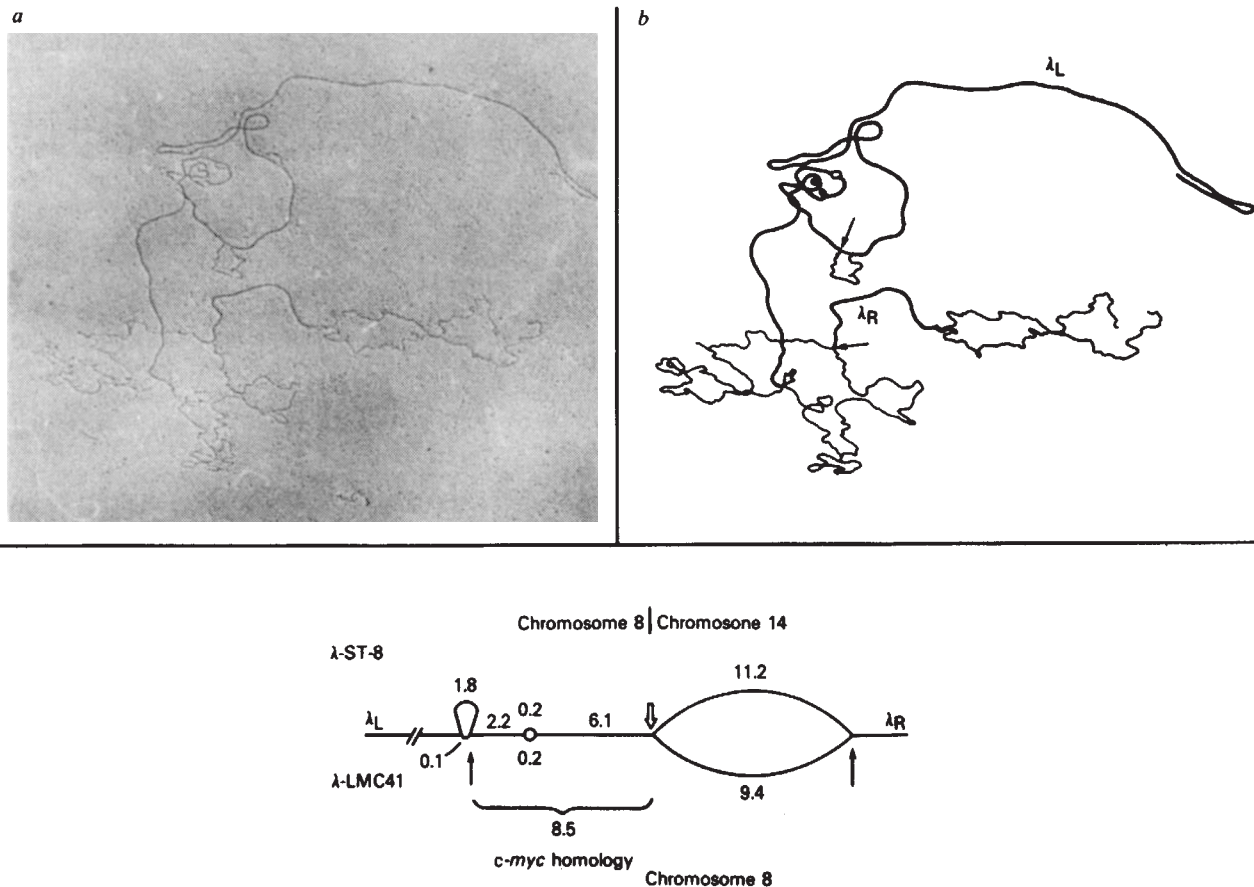


Fig. 3 Heteroduplex comparison of normal and translocated *c-myc* locus. Analysis was performed as previously described⁴⁰ using λ -LMC-41, a clone containing the human *c-myc* locus⁷ and λ -ST-8, one of the clones shown in Fig. 1. In both λ clones the *c-myc* sequences are oriented in the same direction with respect to phage arms and both begin with nearly the same site downstream from *myc* adjacent to the left arm of phage λ vector. *a*, Photograph of a typical heteroduplex molecule. $\times 38,000$. *b*, Tracing of the photograph in *a*. Arrows indicate the junctions between phage vector arms and cloned genomic inserts. Located directly above the arrow at the left arm junction is the 0.2-kb substitution loop discussed in the text. *c*, Schematic drawing of the heteroduplex molecule. Measurements are in kb and are an average of 10 molecules with a standard deviation of less than 7% for each measurement > 1 kb. The arrows indicate the phage vector-genomic insert junctions. The left side of the molecule which is mostly duplex DNA results from hybridization between the *c-myc* sequences and downstream flanking regions. The 0.2-kb substitution loop is located in the 3'-flanking region. The large substitution loop in the right represents 5' *myc* and flanking sequences of LMC41 and IHC $_{\mu}$ switch, C_{μ} and downstream sequences. Size in kb is determined by comparison with internal single- and double-standard marker molecules.

inversion or replacement with a comparable size fragment. Whether this replacement is present in the native ST486 *c-myc* gene or only on the translocated chromosome is unknown. The significance of this structure will be investigated in future studies. We conclude that part of the first *c-myc* intron, the second and third exons, but not the first exon, are translocated into the IHC $_{\mu}$ switch region in this case of Burkitt lymphoma.

The notion that the t(8:14) chromosomal translocation is a reciprocal event has been suggested by cytogenetic studies³⁻⁵, yet never demonstrated at the molecular level. Screening of the ST486 library with a probe extending beyond the chromosomal breakpoint was aimed also at isolating the reciprocal translocation point. Clone λ -ST-20, which was preliminarily identified as bearing rearrangements different from the other clones, was characterized by hybridization to different probes from both the *c-myc* and the IHC $_{\mu}$ loci. With respect to *c-myc*, only probes containing the first exon (pMC415PP, MC41HS5) hybridized to λ -ST-20 DNA, whereas sequences from the first intron, second and third exon were not present (see Fig. 4). Accordingly, the IHC $_{\mu}$ locus is conserved in its 5' sequences (residual portion of the switch region, at least part of *J* region and sequences between switch and *J*), but has lost all the C_{μ} domain. Since the *myc* and IHC $_{\mu}$ sequences present in λ -ST-20 are the ones missing in the four clones described above and vice versa, this clone meets the structural criteria for representing the reciprocal translocation, presumably on chromosome 8q-. The region of

translocation was mapped to a 5-kb segment (11-16 kb on the map) within which the designated probes hybridized. However, to the left of this 5-kb region (11-16 kb on the map) the *Hind*III, *Bam*HI, *Eco*RI and all other sites do not align with the restriction map of the *J* region and upstream flanking portion of the IHC $_{\mu}$ locus. To the right of the 5-kb region (16-17 kb on the map) the *Xho*I and *Bam*HI sites do not correspond to the known maps of several *c-myc* loci. These findings indicated that a more complex rearrangement than two single breaks and rejoining were involved in this example of a chromosomal translocation. Further studies designed to determine the chromosomal origin (chromosomes 8, 14 or other) of rearranged genomic regions should be useful to understand fully the complete architecture of this chromosomal translocation.

Thus, data presented here provide direct evidence that the t(8:14) translocation is reciprocal. The first translocation site, joining the second and third *c-myc* exons with the C_{μ} domain of the IHC $_{\mu}$ locus, occurs within the IHC $_{\mu}$ switch region and the first *c-myc* intron, and is likely to be located on chromosome 14q+, as previous studies by hybridization analysis of somatic cell hybrids have shown that the C_{μ} domain is retained on chromosome 14 and the *c-myc* gene (identified by its two coding exons) is translocated in other UBL cell lines^{8,20}. Further studies combining molecular and cytogenetic studies on the same cells will help to confirm this observation and to define the orientation of *c-myc* and IHC $_{\mu}$ loci with respect to the centromere of their

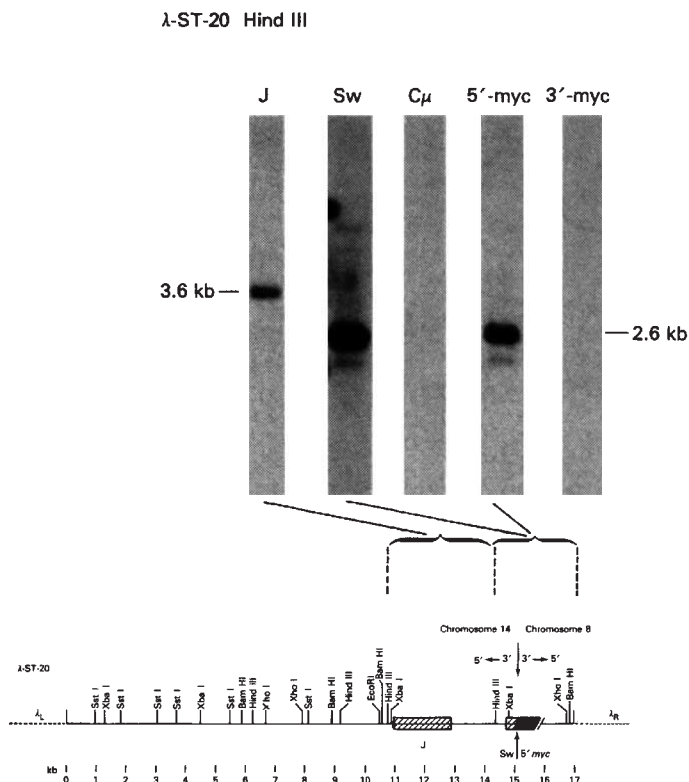


Fig. 4 Genomic organization of the reciprocal translocation site. Cloned λ -ST-20 was analysed essentially as described in Fig. 1 for the other clones isolated. The upper panel of the figure shows identical blots of the λ -ST-20 DNA cleaved with *Hind*III and hybridized to different *IHC μ* and *myc* probes. 5' *myc* probes include clones pMC415PP and MC41HS5 (see Fig. 1) which hybridize to the same 2.5-kb band. 3' *myc* probes, which did not hybridize to λ -ST-20, include clone pMC413RC and the 0.6 *Sst*I–*Sst*I fragment spanning the first *c-myc* intron, and the 0.4-kb *Pst*I–*Pst*I fragment spanning part of the second exon (see Fig. 1a). *IHC μ* probes are as in Fig. 1b. The switch probe hybridized to the same 2.6-kb *Hind*III band which contains 5' *myc* sequences. The *J* region probe hybridized to the adjacent 3.6-kb band. The faint bands in the Sw and 5'-*myc* lanes do not correspond to bands visible in the gel by ethidium bromide staining of the DNA and are artefacts. The lower panel shows the restriction enzyme map of the 17-kb λ -ST-20 insert. The breakpoint in the *J* region is determined by the discordance from that point with the known map of the *IHC μ* locus. The point of discontinuity in the *myc* flanking region is not determined. *Xho*I and *Bam*HI sites at the location shown are not present in the germ-line 5' *myc* flanking region².

respective normal and rearranged chromosomes. While measurements of segments involved in the recombination indicate that no significant loss of genomic material has occurred at the combination site, an exact estimate of the nucleotide levels awaits complete sequencing of the second translocation site. However, as the germ-line sequence of both the *c-myc* first intron and switch μ regions are known, our data allow us to rule out a recombination event mediated by simple sequence homology.

The second translocation site, presumably on chromosome 8q–, contains the reciprocal recombination event, as residual 5' *c-myc* sequences are joined to *IHC μ* switch and *J* sequences. Although analysis of this site provides evidence at the molecular level for a balanced reciprocal translocation, preliminary analysis of flanking sequences suggests that further rearrangements have occurred on both sides of the region containing the switch–*myc* recombination. The significance of these findings is not known and several possibilities must be considered, including a recombination of *J* sequences with upstream immunoglobulin

sequences and the involvement of a third chromosome as described in other cases²⁴. These additional recombinations may have occurred at the same time as the original translocation or subsequently during cell line culture or during cloning procedures.

The identification of translocation sites within the *c-myc* *onc* gene and their relationship with *IHC μ* sequences bear important implications for the regulation of *c-myc* expression and for the structure of *myc* protein product. First, as the translocation in ST486 truncates the *c-myc* gene within its first intron, a normal mRNA would not be generated from the translocated gene. However, the location of the breakpoint within the 5' portion of the *c-myc* *onc* gene would allow the translation of a normal *myc* polypeptide, as the coding potential of the *c-myc* gene is confined to the second and third exons, apparently left intact by breakage and translocation. Second, the position of *IHC μ* regulatory elements, particularly enhancer elements²⁵, which may affect *c-myc* expression, can now be mapped in relationship to different rearranged *myc* sequences. A tissue-specific enhancer element has recently been mapped in the segment between the switch and *J* regions of the mouse *IHC μ* locus^{26–28} and perhaps is conserved in the human genome as are most of the functional domains of the *IHC* locus²⁹. However, the data presented here preclude the presence of this enhancer-containing region on the same chromosome with the coding portions of the *c-myc* gene. In ST486 the enhancer region is located in proximity to the first noncoding *c-myc* exon at the reciprocal 8q– translocation site in the conserved *J*-switch segment. Consistently with both these observations two *myc* RNA species are detectable in ST486 cells and in other UBL cells carrying a t(8:14) translocation interrupting the *c-myc* gene in its first intron. A 2.3-kb mRNA species contains transcripts from the second and third exon and is transcribed at relatively low levels. A second 1.1-kb species containing transcript from the first exon is expressed at much higher levels as an effect of the nearby enhancer sequence on chromosome 8q– (R.D.F. *et al.*, manuscript in preparation).

Deletion of the first exon or 5' flanking sequences from the normal *myc* transcript may alter the regulation of expression of this *onc* gene, as these 5' sequences are absent in other situations where *myc* expression is associated with oncogenesis. These situations include the transduction of *myc* into a retrovirus genome to form a viral *onc* gene³⁰ or the insertion of a retroviral genome into the 5' *myc* region in some avian leukemia virus-induced lymphomas³¹. However, note that in only approximately one-third of UBLs is the chromosomal breakpoint located within the *c-myc* locus, whereas in the rest of UBL displaying the t(8:14) translocation the breakpoint appears to be located further upstream from the translocated *myc* gene (R.D.F. *et al.*, unpublished). Moreover, we have recently characterized a case of Burkitt lymphoma carrying a t(8:22) translocation which leaves the *c-myc* locus on chromosome 8, the breakpoint most probably occurring downstream from *c-myc*³². These data, taken together with a lack of evidence for a generally increased level in *c-myc* transcription in UBL (ref. 33 and R.D.F. *et al.*, manuscript in preparation) may suggest that the common denominator linking *c-myc* expression and UBL pathogenesis may be a lack of regulation as opposed to enhanced transcription as previously suggested³⁴. Different rearrangements affecting region q24 on chromosome 8 may cause constitutive *c-myc* expression which may be associated with cell proliferation³⁵, whereas normal terminal differentiation would require *c-myc* suppression as seen *in vitro* in a human leukaemia cell line³⁶.

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The protein products of the *myc* and *myb* oncogenes and adenovirus E1a are structurally related

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Structural and functional homologies have been found among proteins encoded by several retroviral oncogenes, demonstrating the existence of families of these genes¹. Because the retroviral oncogenes have cellular homologues^{1,2}, the existence of similar families among these 'cellular oncogenes' is also implied (for a review, see ref. 2). Cellular genes belonging to these families have been found in such evolutionarily distant species as humans^{3,4}, fruit flies^{5,6}, nematodes⁵ and brewer's yeast (*E. Scolnick and S. Reed, personal communications*), consistent with the hypothesis that these genes have evolved from a small number of ancestral sequences². We extend these observations by showing here that the proteins encoded by the oncogenes *myc*, *myb* and adenovirus E1a are structurally related. Our findings suggest that oncogenes of RNA and DNA tumour viruses may in at least some instances share evolutionary origins and function according to common principles.

Having obtained the nucleotide and deduced amino acid sequences of the retroviral oncogenes *v-myc*⁷ and *v-myb*⁸, we

undertook a computer search for related proteins among the current entries in the Protein Sequence Database of Dayhoff⁹. The search revealed that the proteins encoded by *v-myc* and *v-myb* each contained several short strings of amino acids that were either identical or very similar to strings of amino acids in the proteins encoded by the E1a region of adenovirus type 12 (Ad12)¹⁰ (data not shown). E1a is a transcriptional unit that resides at the leftward extreme of the adenovirus genome and that, when acting alone, induces a 'partially transformed' phenotype whose principal feature is extended growth potential ('immortalization')¹¹. These initial results indicated only that similarities of uncertain significance might exist. To evaluate further the relationships among the various proteins, we used the matrix comparison devised by McLachlan¹². The strength of the comparisons was enhanced by using the sequences for the related but non-identical E1a regions of adenoviruses type 5 (Ad5)¹³, type 7 (Ad7)¹⁴ and type 12 (Ad12)¹⁰ and the sequences for *v-myc*⁷ and human *c-myc*¹⁵.

Comparison of the sequences, using the data for both adenovirus 12 E1a and *v-myc* as reference standards (Fig. 1), indicated appreciable homology among the E1a proteins of Ad5, Ad7 and Ad12, as anticipated, intermediate homologies between the *myc* and E1a proteins, and weak but detectable homology between *myb* and both *myc* and E1a. The homology between *myc* and *myb* is slightly more extensive than that between *myc* and the E1a proteins of Ad5 and Ad7.

We then arranged the sequences to obtain maximum alignment of their homologous regions (Fig. 2). The matrix comparisons indicated that we would be looking for distant

Fig. 1 Statistical analysis of relatedness. The sequences of proteins encoded by E1a, *myc* and *myb* were compared using the matrix procedure of McLachlan¹², using a VAX 11/780 computer. The sequences encoded by 12S mRNAs were used for the E1a proteins. Two reference sequences were used for the comparisons, sequence of Ad12 E1a (left panel) and the sequence of *v-myc* from MC29 virus (right panel). Standards for unrelated sequences with identical amino acid compositions were obtained by comparing the sequences with randomly scrambled versions of themselves. The results of this comparison give the leftward-most curves in the plots. The complete identity obtained when a sequence is compared against itself is defined by the rightward-most curves. Curves describing proteins related by non-identical sequences fall between the two extremes. The sequences used in the comparisons were *v-myb* (A), *c-myc* (B), Ad5 E1a (D) and Ad7 E1a (E). Curve C (*v-myc* compared with Ad12 E1a or Ad12 E1a compared with *v-myc*) is the same in both panels. Probability is plotted as a function of score. The actual sequences used for the matrix comparison are shown in Fig. 2a.

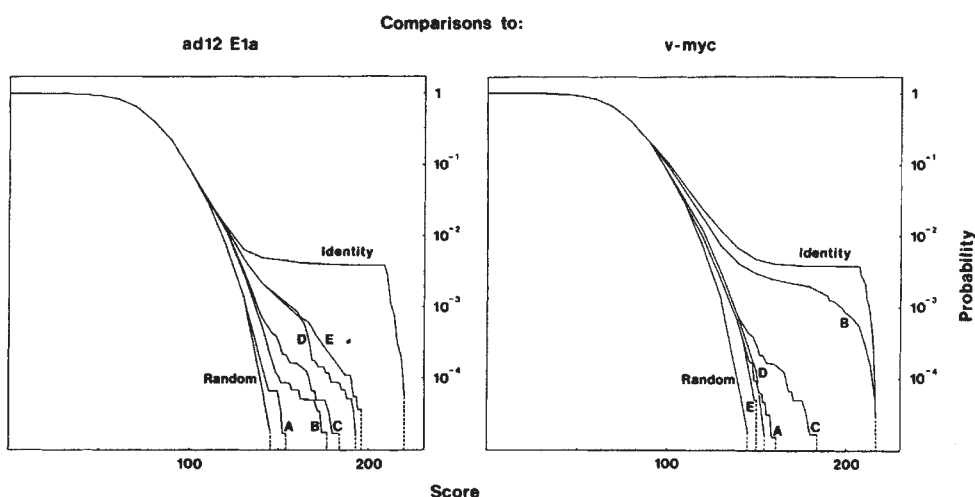


Fig. 2 Alignment of related sequences. The deduced amino acid sequences for E1a (the smaller proteins, encoded by 12S mRNAs), *myc* and *myb* were arranged to achieve maximum alignment of homologous regions (a). First, the three E1a proteins were aligned among themselves, and *v-myc* was aligned with human *c-myc*. Second, strings of homologous sequences in the E1a and *myc* proteins were used as guides to align the two groups of proteins against each other. Third, this alignment was in turn aligned with *v-myb*. Each alignment was accomplished as follows. First, occasional regions of identical strings of amino acids were used to define fixed positions in the alignments (examples include residues 14-20, 61-63, 97-100, 117-119, 172-180, 243-247 and 271-288). Regions between the strings of identities were then aligned by inspection, using the string matching data from the matrix comparisons to identify regions of similar sequence. Judgements of similarity are based on statistical analysis of substitution frequencies for pairs of amino acids in 16 protein families¹². As the alignment proceeded, closely related sequences (the family of E1a proteins on the one hand, the *myc* proteins on the other) were used to locate areas where variation in sequence length (insertions or deletions of sequence) is tolerated, and to evaluate the significance of individual amino acid substitutions. For example, in areas where it was difficult to match type 12 E1a and *v-myc*, the other related sequences often provided guides to alignment. The initial alignment of all seven available sequences was re-checked several times to maximize the alignments among all of the sequences. The final result is illustrated in the figure. a, Aligns the complete sequences of the smaller E1a proteins^{10,13,14}, residues 97-372 of *v-myc*⁷, residues 102-386 of human *c-myc*¹⁵, residues 63-334 of *v-myb*⁸ and residues 63-334 of chicken *c-myb*⁸. The residues deleted in the Ad5 mutant *d/311*³⁶ are indicated by the bar above the Ad5 E1a sequence (residues 206-226). b, Aligns the additional domain in the larger E1a proteins with homologous regions from the other proteins. Capital letters denote the amino acids found in the larger but not the smaller E1a proteins. For Ad5 E1a, the sequence unique to the protein encoded by the 13S mRNA follows the Gly at alignment position 180. A 20-residue gap must be inserted between this Gly and the first Glu of the unique sequence to align it with the homologous sequence of Ad12 E1a. Also shown are the amino acid sequences from the larger E1a proteins of the Ad5 host-range mutants H5hr440³⁵, H5in500³⁶ and H5hr1³⁴. H5hr440 terminates at the Glu shown in the lower panel. H5in500 is an insertion/frameshift mutant that alters the sequence of the unique region and terminates at the Ile shown. H5hr1 is a deletion/frameshift mutant that terminates at the Ile shown. The mutations in H5in500 and H5hr1 affect only the products of the 13S mRNAs. H5hr440 also contains a mutation that prevents splicing of the 12S mRNA, so that only the mutant protein is made. In all of the alignments, asterisks denote identities between amino acid residues or substitutions that occur with greater than average frequency among related proteins as established by McLachlan¹²; boxes are used to emphasize regions where comparison of both Ad12 E1a with *v-myc* and *v-myc* with *v-myb* reveal strings of homology whose likelihood exceeds average expectation, again according to the scores assigned by McLachlan. The direct repeat in the amino terminus of *myb* is shown in c. The sequence begins with the first amino acid of the *myb*-specific region of AMV. This duplication is also present in the amino terminus of *c-myb*⁸. The amino acid differences in *c-myb* are indicated above and below the *v-myb* sequences. The positions of the splice junctions in *c-myb* are shown by arrowheads.

relationships, presumably sufficient to conserve tertiary structure and homologous functions of proteins, but not involving extensive strings of identical amino acids. We therefore used a multistep strategy that first aligned the proteins already known to be related (the E1a proteins as one example, the viral and cellular *myc* proteins as another), then used the homologies between the most closely related members of the two groups to align both sets of sequences. This method had the advantage of making conserved sequences more apparent and providing information about allowable substitutions and regions of variability within each group.

The final alignments of the various amino acid sequences revealed regions of homology distributed along the entire lengths of the proteins. The homologies involve conservative substitutions of amino acids more frequently than identical residues. Although relationships are most apparent between

a Alignment of Sequences

	10	20	30	40	50	60
ad5 E1a	MHTICGGGV	I--TEEMVL	LDQLLEEV	ADNLPPP-SH	FEPPTLHLY	DL--DVAF
ad7 E1a	MHLRLFLPE	ITSSFTGIE	ILFVWNTLM	GDPPEPVOP	FDPPTLHLY	DL--EVDGF
ad12 E1a	MHTMT-PLV	L--SYGEADD	ILHLVDNFF	--NEVPSDDL	Y-VPSLYELY	DL--DVESAG
<i>v-myc</i>	-V-TELLGGDM	VNQSFICDD	DESFVKSI	QDCMVSQFA	A-AKLEKVS	EKLATYQAS
<i>c-myc</i>	-V-TELLGGDM	VNQSFICDD	DESFVKSI	QDCMVSQFA	A-AKL--VS	EKLATYQAA
<i>v-myb</i>	-WHNHLNPEV	KTSWTEED	RITYQAKRL	GNR-WAETAK	L-LPGRTDVA	VK--WHNSTM
<i>c-myb</i>	-WHNHLNPEV	KTSWTEED	RITYQAKRL	GNR-WAETAK	L-LPGRTDVA	TK--WHNSTM
	70	80	90	100	110	120
ad5 E1a	--DPNKEAV	SQI--PFD	3--VMLAVGE	GIDLTFPPA	--PQSPPEPP	LSRQPPQPE
ad7 E1a	--DPNKEAV	NGF--PFD	3--MLAADE	GDLNPPPET	SVTHPGVVS	GRGOKLPDS
ad12 E1a	--DPNKEAV	NEF--PFE	3--LLAASE	GL-FLPEPV	--LSVVC--	EPIGGECHPL
<i>v-myc</i>	RREGGPAAS	RPQFPSPF	PPAGPAASA	GL-YLHDLGA	--AAADCI--	DPSVV--FPFP
<i>c-myc</i>	RKDSGSPHFA	RHGS--	--VCSTL	SL-YLQDLGA	--AASECI--	DPSVV--FPFP
<i>v-myb</i>	RRKVEGEGY	QESSKAGPS	ATTOFQKSH	IMAFANPPA	--DPLPQAG	APLQSDYFPY
<i>c-myb</i>	RRKVEGEGY	QESSKAGPS	ATTOFQKSH	IMAFANPPA	--DPLPQAG	APLQSDYFPY
	130	140	150	160	170	180
ad5 E1a	RALGPSMPN	LV-----	-----	--PEVIDL	TCM--EA-GF	PPSDDDEERG
ad7 E1a	-----	-----	-----	--GAEMDL	RCY--EE-GF	PPSDDDEERG
ad12 E1a	-----	-----	-----	--PEDMDL	LCT--EM-GF	PPSDDDEERG
<i>v-myc</i>	LSRAPRAAP	-----	-----	--FQANPAAL	LCT--DTPPT	PPSDDDEERG
<i>c-myc</i>	LWDSSPKSC	ASQDSSAFSP	SSDLSLSSTE	SSPQSSPEPL	VHETETPT	PPSDDDEERG
<i>v-myb</i>	HIAEPQNV--	-----	-----	--PGQIPYV	ALMI--NIENY	PQPAATAAOR
<i>c-myb</i>	HIAEPQNV--	-----	-----	--PGQIPYV	ALMI--NIENY	PQPAATAAOR
	190	200	210	220	230	240
ad5 E1a	-----PVS-	-EPEPEPEP	PEPAFTPEP	-KMAPILTR	PTSPVSRCH	SSTDSC-DSG
ad7 E1a	-----ST	-HTAVN	EDVKAAADFP	-KLDCEPLG	HGCFVDDDS	SPSDSTT--
ad12 E1a	-----ST	-HTAVN	EDVKAAADFP	-KLDCEPLG	HGCFVDDDS	SPSDSTT--
<i>v-myc</i>	DEDEIDVTL	AEAMESST	ESSTAESST	CKPHSPVLV	KRCHV--	-----
<i>c-myc</i>	DEDEIDVTL	AEAMESST	ESSTAESST	CKPHSPVLV	KRCHV--	-----
<i>v-myb</i>	HYTDED-PEK	KKR--	IKEL	ELLMTSTNE	LKQQA-LPT	-----
<i>c-myb</i>	HYTDED-PEK	KKR--	IKEL	ELLMTSTNE	LKQQA-LPT	-----
	250	260	270	280	290	300
ad5 E1a	PSNTTPE	-----	HPVPLCPXK	FVAVRGGRR	Q-AVEICEDL	L--NEPOQ--
ad7 E1a	-----	-----	IPVKKPKGR	PAVKLELDL	EGG-DQPLDL	STKLPLRQ
ad12 E1a	-----	-----	IPVKKPKGR	PAVKLELDL	EGG-DQPLDL	STKLPLRQ
<i>v-myc</i>	NTHQHNY	-A	APPSTKVEP	AAKRLKGLSV	RVLKQSNRR	KCSPPTLDS
<i>c-myc</i>	NTHQHNY	-A	APPSTKVEP	AAKRLKGLSV	RVLKQSNRR	KCSPPTLDS
<i>v-myb</i>	QHNTANY	-----	DMTSTSDNA	PV-SCLEGH	HCTPSPFPV	GCLPEESA-S
<i>c-myb</i>	QHNTANY	-----	DMTSTSDNA	PV-SCLEGH	HCTPSPFPV	GCLPEESA-S
	310	320	330			
ad5 E1a	PLD-LSCK-R	P-RP	-----			
ad7 E1a	PVD-LSVK-R	P-RCH	-----			
ad12 E1a	-----	-----	-----			
<i>v-myc</i>	-----	VLER	Q-RRNELKLR	FFA-LRDQIP	EVAN--	-----
<i>c-myc</i>	-----	VLER	Q-RRNELKLR	FFA-LRDQIP	ELEN--	-----
<i>v-myb</i>	PARCMIVHQS	NILDNVKNLL	EFATLLOLD	SFLN-	-----	-----
<i>c-myb</i>	PARCMIVHQS	NILDNVKNLL	EFATLLOLD	SFLN-	-----	-----

b Alignment of Sequences Unique to Large E1a Proteins

H5hr440 E1a	---EFP	VLDYVEHP	IPOTVAGLVI	ITGIGRTQI	LCVRFAI
H5in500 E1a	---EFP	VLDYVEHP	HOCNSCHYHR	RNTOTQILCV	RFAI
H5hr1 E1a	---EFP	VLDYVEHP	HOCNSCHYHR	RNTOTQILCV	RFAI
ad5 E1a	---EFP	VLDYVEHP	HOCNSCHYHR	RNTOTQILCV	RFAI
ad7 E1a	---EFP	VLDYVEHP	HOCNSCHYHR	RNTOTQILCV	RFAI
ad12 E1a	---EFP	VLDYVEHP	HOCNSCHYHR	RNTOTQILCV	RFAI
<i>v-myc</i>	---EFP	VLDYVEHP	HOCNSCHYHR	RNTOTQILCV	RFAI
<i>c-myc</i>	---EFP	VLDYVEHP	HOCNSCHYHR	RNTOTQILCV	RFAI
<i>v-myb</i>	---EFP	VLDYVEHP	HOCNSCHYHR	RNTOTQILCV	RFAI
<i>c-myb</i>	---EFP	VLDYVEHP	HOCNSCHYHR	RNTOTQILCV	RFAI

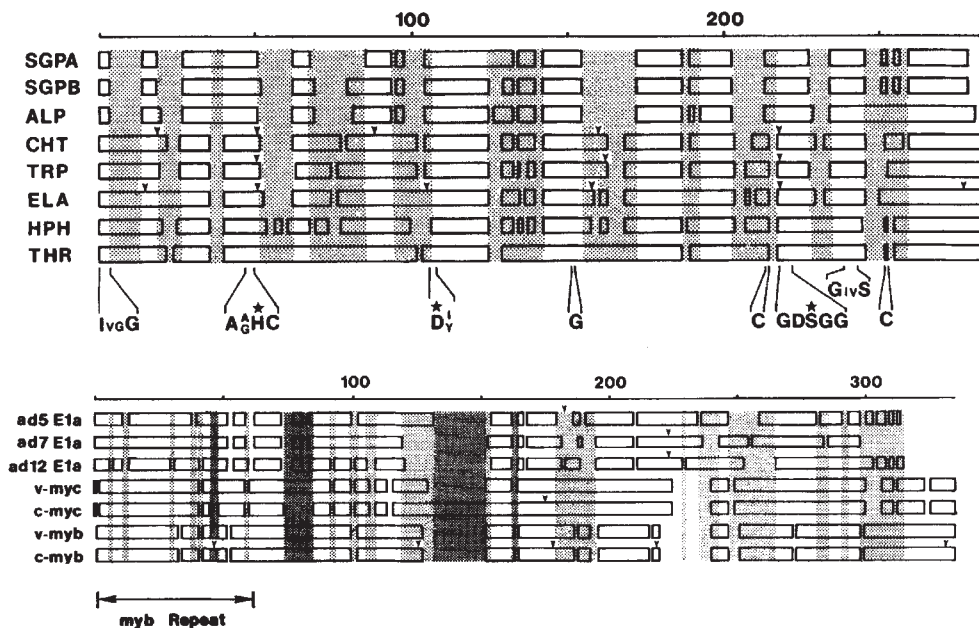
c Direct Repeat in the N-terminus of myb

	1	10	20	30	40	50
NRTDVQCQHR	WQVLPNPEL	KOPVTEKEDQ	RIVHVKYK	PKRWSDIAKH	LKGRIG	
-----	-----	-----	-----	-----	-----	
KQCRER	WHNHLNPEV	KTSWTEED--	RITYQAKRL	GNRWARTAKL	LPGRTDNAV	
	60	70	80	90	100	110

myc and Ad12 E1a, and between *myc* and *myb*, there are regions where E1a is more closely related to *myb* than to *myc*. The utility of our strategy is illustrated by the relationships we were able to perceive between *myc* and *myb*. These became apparent through the recognition of sequences conserved in both the E1a proteins and the *myc* proteins. Thus, the regions in which E1a and *myc* are most closely related are generally also related to *myb*. Once perceived, the relationships between *myc* and *myb* proved to be as great as those between *myc* and Ad5 or Ad7 E1a.

The number of identical amino acids in pairwise comparisons between proteins from different families (*myc*, *myb* and E1a) ranges from 15 to 21%. Although the overall number of identities is not high, it is significant that they tend to cluster similarly among all of these proteins. The number of identities compares favourably with data for distantly-related members of well-established families such as the globins¹⁶, cytochromes¹⁶, dehydrogenases^{17,18}, repressors¹⁹ and serine proteases^{20,21}, in which homologous regions were defined much as we have done here.

Fig. 3 Topographical homologies. **a**, Gap positions in alignments of serine proteases. The amino acid sequences of eight bacterial and eukaryotic serine proteases, and haptoglobin⁴⁵ have been redrawn to represent contiguous residues in the alignments as blocks. The sequences shown are chymotrypsin (CHT), trypsin (TRP), elastase (ELA), haptoglobin (HPH), thrombin (THR), *S. griseus* proteases A and B (SGPA, SGPB) and α -lytic protease (ALP). The consensus regions of length variation are emphasized by shading. The positions of splice junctions in the mRNAs are mapped onto the amino acid sequences (indicated by arrowheads). Amino acids that are invariant in these proteases are shown in bold type below the block alignments. The number of invariant residues, though small, is larger than the number of invariant residues (3/170) in the globin family¹⁶. The active site residues are indicated with stars as a guide to topography. Haptoglobin (HPH) differs from the consensus residues in having TAKN instead of AAH*²⁰ and GDAGS instead of GDS*²⁰. All of the proteases shown in the block alignments have related tertiary structures²⁰. The gaps shown in the alignment map to loops in the tertiary structures^{20,22}. Note that the conserved residues are widely distributed along the sequences. **b**, Gap positions in alignments of the *myc*, *myb* and E1a proteins. The sequence alignments in Fig. 2a have been redrawn to represent contiguous residues by blocks. The 17 gaps that occur in the alignment of the E1a proteins as a group, and that are not due to alignment with *myc* or *myb*, are shown by the lightly shaded areas. The three gaps that occur in the alignment of the *myc* sequences are indicated by darkly shaded areas. These shaded areas reflect regions of polypeptide length variation (that is, insertions or deletions of sequence) within the *myc* or E1a families. The positions of splice junctions are indicated by arrowheads. The position of the direct repeat in the *myb* sequences (Fig. 2c) is also indicated.



While performing the alignments, we discovered a previously unappreciated duplication of sequence in the amino-terminal domain of *v-myc* (Fig. 2c). The rightward unit of the repeat is included in the alignments illustrated by Fig. 2a, the leftward unit is not. Analogous repetitions are not apparent in any of the other proteins studied here.

To achieve the alignments illustrated by Fig. 2, it was necessary to place gaps at various positions in the amino acid sequences. Insertions and deletions of amino acid sequence occur frequently in evolutionarily related proteins, and are observed as gaps in protein sequence alignments¹⁶. For example, numerous gaps are needed to align the amino acid sequences of the serine proteases (Fig. 3a). In general, the gaps occur at similar locations in the various proteins, thereby indicating regions where variations in sequence length can be tolerated²²⁻²⁴.

Figure 3b illustrates the placement of gaps in the aligned sequences of the E1a, *myc* and *myb* proteins. It is apparent that there is a general (but not inevitable) concurrence between the positions of gaps in the related E1a proteins and the proteins encoded by the two retroviral oncogenes. The frequency of gaps is comparable with that observed among distantly related members of the serine protease family. The manner in which gaps in the alignments reflect regions of permissible structural variation is dramatized by the need to use two relatively large gaps to align satisfactorily the very closely related sequences of *v-myc* and *c-myc*, and the need to use 17 gaps to align the E1a sequences (see Figs 2a, 3b). We conclude that the consistent disposition of gaps along the aligned sequences of E1a, *myc* and *myb* adds to the credibility of the alignments and suggests that the proteins have related structures.

The disposition and number of gaps in the sequence alignments are also consistent with other features of these proteins. Hydrophobicity calculations²⁵ indicate that all of the proteins analysed here are predominantly hydrophilic (data not shown). In addition, the amino acid sequences are uncommonly rich in proline, glycine and serine, residues that tend to disrupt regular secondary structure. These observations suggest that the tertiary structures of these proteins probably contain many solvent-accessible loops. In other protein families, such structures tend to be hotspots for insertions and deletions^{23,24}.

ad12 ProCysSerAspSerGluAspGluGlnAspGluAsn
CCTGTAGCGATTGCGAAGACGACGACGACGAC
* * * * *
ACCAGCAGCGACTCGGAAGAAGACGACGACGAC
v-myc ThrSerSerAspSerGluGluGluGlnGluGluAsp

ad12 ProValProGlnArgValThrGlyArgArgArgCys
CCTGTGCTTCA-CCGGGTGACGGGAGGCGTAGATG
* * * * *
AGCGT-CCTCAACAGATCAACGACGACGACGAC
v-myc ArgVal-IleuLysGlnTleSerAsnAsnArgLysCys

Fig. 4 Nucleotide sequence homologies between *myc* and Ad12 E1a. The nucleotide sequences of *v-myc* and Ad12 E1a corresponding to positions 171-192 and 271-282 in Fig. 2 are shown. Asterisks indicate identical nucleotides. The nucleotide sequence of chicken *c-myc* is identical to *v-myc* in these regions⁴⁰.

Recent work has indicated that exon splice junctions frequently map to amino acid sequences located on the surfaces of native proteins, frequently within or near regions of length variation in related proteins²¹. The splice junctions for E1a, *myc* and *myb* are located in rough accord with this principle (see Fig. 3b).

The unexpected structural homology between proteins encoded by oncogenes of RNA tumour viruses and DNA tumour viruses raises the question of the origin of this relationship. The issue of convergence versus divergence as the basis of structural relationships between proteins is usually not resolvable unless the number of identical amino acids is commandingly large²⁶. However, the premise that it is easier to duplicate and then modify genetic components rather than to assemble new genes *de novo* from random beginnings has led Doolittle to propose that all proteins have evolved from a small number of ancestral sequences²⁶. In the case of *myc* and *myb*, the small number of identities and lack of information about higher order structure or function make any decision between convergence and divergence necessarily subjective. For *v-myc* and Ad12 E1a, however, we can show that the relationship is unlikely to be due solely to convergence.

Because convergence is defined as structural similarity resulting from functional requirements, its effect must be due to selection at the protein level. Figure 4 shows the nucleotide sequences of Ad12 E1a and *v-myc* in two widely separated regions of amino acid sequence homology. The nucleotide sequences show many identities in these regions; indeed, more

than would be expected on the basis of amino acid similarities. For example, the two pairs of aligned serine codons in the upper panel are identical, even though there are 72 possible combinations. In addition, there are strings of identities in the nucleotide sequences that are apparent when two gaps are introduced (lower panel). These identities are not in the reading frame, and would not be expected to occur through convergence. However, such identities could be accounted for by two frame-shift mutations during evolution of one of the sequences. The unexpected similarities in the nucleotide sequences of Ad12 E1a and *myc* suggest the existence of a common ancestral sequence. The origin of such a sequence could be either common ancestry of adenoviruses and eukaryotic cells or recombination between ancestral viral sequences and cellular *myc* sequences.

Several features of the oncogenes we have compared sustain our conclusion that these genes are all relatives of one another. First, the proteins encoded by *myc*^{27,28}, *myb* (K.-H. Klempnauer, personal communication) and E1a²⁹⁻³¹ are all located in the nucleus of transformed cells. Second, it has recently been shown that E1a and *v-myc* serve similar establishment functions in transformation of cells in culture^{32,33}. We learned of these results only after presenting our arguments for a structural relationship between *myc*, *myb* and E1a. Third, genetic analyses have shown that for Ad5, the larger E1a protein encoded by the 13S mRNA is essential for the function of E1a in cellular transformation³⁴. By contrast, there is evidence that the smaller of the two E1a proteins may be sufficient for transformation by Ad12²⁹. In accord with this contrast, a string of homologous residues shared by *v-myc* (residues 208-225 in the upper portion of Fig. 2) and the smaller E1a protein of Ad7 and Ad12 is contained instead in the larger E1a protein of Ad5 (Fig. 2b). Ad5 mutants whose lesions are within this region of homology, such as H5hr440³⁵ or H5in500³⁶ (Fig. 2b), are transformation defective. In contrast, the E1a mutants H5hr1³⁴ (Fig. 2b) and d1311³⁷ (Fig. 2a) retain transformation ability, although the phenotype is cold-sensitive in cells transformed by H5hr1^{38,39}.

There are nevertheless significant differences in the biological effects of these oncogenes. For example, *v-myc* is capable of transforming avian fibroblasts and macrophages, whereas *v-myb* can transform avian macrophages but not fibroblasts¹. The E1a proteins by themselves do not cause transformation, but can immortalize primary rat embryo fibroblasts (REFs)¹¹. Also, *v-myc* is apparently unable to transform mammalian cells, although its effect on primary REFs is similar to E1a (E. Ruley, personal communication). While there are clearly important differences between these oncogenes, the existence of homologous sequences in homologous positions raises the possibility that the tertiary structures of these proteins have similar features that in turn underlie related functions.

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Transcription enhancer identified near the human C_{μ} immunoglobulin heavy chain gene is unavailable to the translocated *c-myc* gene in a Burkitt lymphoma

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In Burkitt lymphoma the *c-myc* gene, the cellular homologue of the viral oncogene *v-myc*, has been implicated in the aetiology of this human B-cell malignancy. Burkitt lymphoma cells possess specific chromosomal rearrangements involving the region proximal to the *c-myc* gene and one of the three human immunoglobulin loci¹⁻⁹. The nature of the effect exerted by the immunoglobulin loci on the translocated *c-myc* gene is controversial: whereas some reports have suggested *c-myc* transcription is elevated in Burkitt lymphoma cells¹⁰⁻¹², others have suggested the level of transcription is unaffected by the translocation⁹. Recently, transcription enhancer elements have been identified in the intron between the J_H and C_{μ} segments of the heavy-chain immunoglobulin gene in mice¹³⁻¹⁵. If similar enhancers exist in humans they may lead to increased transcription of the translocated *c-myc* gene and thus contribute to oncogenesis in Burkitt lymphoma. We report here the identification of an enhancer element adjacent to the human C_{μ} gene on normal chromosome 14, but this enhancer does not remain on the abnormal chromosome 14 to which the *c-myc* gene has been translocated in the Burkitt lymphoma cell line Raji. This element is, therefore, not available for control of the translocated *c-myc* gene in this case.

A transient expression assay was used to measure the ability of DNA segments to enhance transcription of immunoglobulin genes. This assay¹⁶ consists of transfecting cloned DNA into myeloma cells followed by S_1 -nuclease mapping analysis of transcribed RNA after a 2-day incubation of the cells. The cloned probes used in this experiment are shown in Fig. 1 and described in Fig. 1 legend. The starting clone, called pXTK-V δ 1,

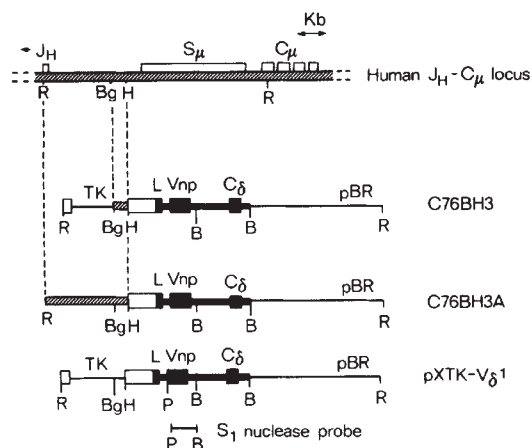


Fig. 1 Diagrammatic representation of plasmid clones used for the transient expression assays. The clones are illustrated in their linear form. The top line represents the human immunoglobulin C_μ locus determined from the clone C76R51²³; note that the *Eco*RI site at the 5' end does not exist in the genome but was created by the original phage cloning protocol²⁴, and is included here since it was used for the subcloning in the plasmid C76BH3A. The clone pXTK-V δ 1 has been described previously¹⁴, and is a pBR322 derivative, containing a thymidine kinase (*tk*) gene (which is not relevant to the study described here), into which mouse DNA was inserted. This mouse DNA consists of a V_H gene derived from a cell-making antibody against 4-hydroxyl-3-nitrophenylacetyl (NP) linked to a C_δ domain together with a poly(A) addition site. The open box immediately upstream of the leader sequence (*L*) indicates mouse DNA containing a promoter for V_H transcription. This mouse DNA segment, therefore, can potentially be transcribed, spliced and terminated to yield mature mRNA. The plasmid C76BH3 is a derivative of pXTK-V δ 1, in which the 0.8 kb *Bgl*III-*Hind*III fragment from the human J_H - C_μ clone C76R51 has replaced the *Bgl*III-*Hind*III fragment in the *tk* portion of pXTK-V δ 1. C76BH3A is a derivative of C76BH3 containing the *Bgl*III-*Eco*RI fragment from C76R51 in place of the remaining portion (*Bgl*III-*Eco*RI) of the *tk* segment. Restriction endonuclease sites: R, *Eco*RI; Bg, *Bgl*III; H, *Hind*III; P, *Pst*I; B, *Bam*HI.

contains an artificially created immunoglobulin gene inserted into a pBR322 vector in such a way that it can be transcribed and correctly spliced into mRNA. Two subclones of pXTK-V δ 1 were derived containing pieces of the intron between the human J_H elements and C_μ gene: clones C76BH3 and C76BH3A (see Fig. 1). At the top of Fig. 1 the DNA fragments from the 5' end of the human C_μ locus used in these constructions are shown.

All three clones were used to transfect the BALB/c mouse myeloma cell line X63 to assess their transcription efficiency. RNA was prepared from the transfected cells and subjected to S_1 -nuclease protection assays using the probe indicated at the top of Fig. 2 and described in Fig. 2 legend. Correctly spliced transcripts would protect a fragment of the probe 350 bases long, corresponding to the V_{np} coding portion only (the part between the arrows in Fig. 2b). Figure 2c shows the result of an S_1 mapping experiment analysed by polyacrylamide gel electrophoresis. Lane 5 shows the intact single-stranded probe and lane 1 shows that the probe is completely digested if not hybridized to RNA. Lanes 2-4 show the results obtained with RNA from cells transfected with pXTK-V δ 1 (lane 2), C76BH3A, which contain the human DNA sequences (lane 3) or C76BH3 (lane 4). RNA isolated from cells transfected with pXTK-V σ 1 failed to yield any detectable S_1 -nuclease protected probe after hybridization, which is consistent with previous results¹⁴ and shows that the functional immunoglobulin promoter present in the clone does not support significant transcription in this assay. However, both C76BH3 and C76BH3A were transcribed efficiently *in vivo* (lanes 3 and 4): in both cases the S_1 nuclease protected fragment was about 350 bases in length, consistent with correct transcription and RNA splicing of RNA made from the V_{np} promoter. Thus, DNA segments between human J_H and S_μ are capable of the enhancement of V_H gene transcription.

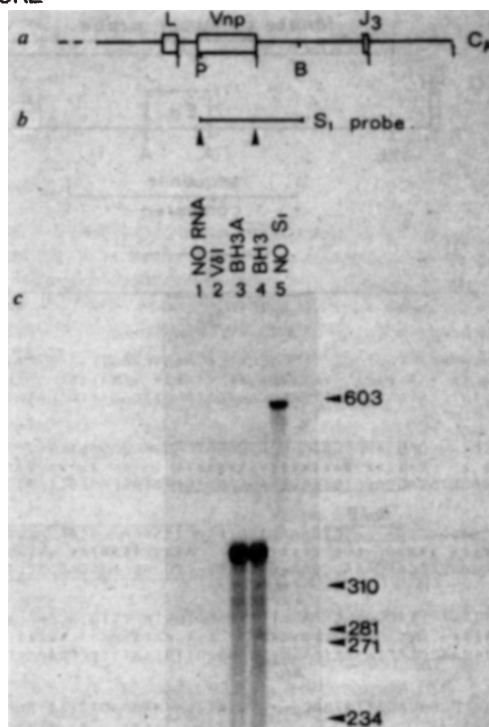


Fig. 2 S_1 -nuclease mapping of RNA from transfected lymphocytes. **a** Shows the clone used to prepare the single-stranded probe, a 570 bp *Pst*I-*Bam*HI fragment, contained within an M13 mp8 vector, extending from the *Pst*I (P) site just within the V_{np} coding region to a *Bam*HI (B) site in the 3' intron¹⁴. **b** Delineates the single-stranded probe, which was labelled by primer extension in the presence of 32 P-dATP followed by elution of the appropriate restriction fragments from an urea/acrylamide gel. The arrows indicate the extent of the expected S_1 -nuclease protected fragment (350 nucleotides long) after hybridization to correctly spliced RNA transcripts. **c**, Acrylamide gel electrophoresis of S_1 -nuclease protected DNA fragments after: lane 1, mock hybridization with no RNA added; lanes 2-4, hybridization of the probe to RNA from cells transfected with: lane 2, pXTK-V δ 1; lane 3, C76BH3A; lane 4, C76BH3; lane 5, untreated P^{32} -labelled probe.

Methods: Plasmid DNA was transfected into X63 mouse myeloma cells in the presence of DEAE-dextran¹³. 5×10^6 X63 cells (harvested at about 5×10^5 cells ml^{-1}) were washed in serum-free DMEM medium and incubated for 30 min at room temperature in medium plus 250 μg ml^{-1} DEAE-dextran and 5 μg plasmid DNA. The cells were subsequently washed with medium plus 10% fetal calf serum (FCS) and resuspended in 10 ml medium, 10% FCS plus 20 μM chloroquine. After 2h at 37°C, the cells were washed in 10 ml DMEM containing 10% FCS and incubated in 15 ml of this medium at 37°C. After 2 days RNA was prepared from the cells as described previously²⁵. RNA samples (10^6 cell equivalents per lane) were hybridized overnight with 50,000 c.p.m. of the labelled single-stranded DNA probe (indicated in **b**) followed by S_1 nuclease digestion²⁶ (0.5 units nuclease in 30 mM sodium acetate, 250 mM NaCl, 1 mM $ZnSO_4$, 5% glycerol for 30 min at 37°C). Digestion mixtures were fractionated in 6% acrylamide gels in the presence of 8 M urea, the gels dried and autoradiographed overnight at -20° with prefogged film. The sizes of DNA fragments were estimated by co-electrophoresis of P^{32} -labelled ϕ X174 DNA digested with *Hae*III (indicated on the right-hand side in **c**).

Furthermore, significant activity was obtained with only the 800 nucleotide fragment present in C76BH3. The presence of the additional human DNA fragment in C76BH3A, however, consistently yielded a 3-5 times greater signal, implying the presence of important sequences in this region. We have been unable to detect any enhancer activity, using a similar assay, when the region between $C_{\gamma}1$ and S_γ was used in place of the C_μ enhancer segments (unpublished data).

We have compared the nucleotide sequences of the human and mouse enhancer regions (Fig. 3) to try to identify functionally important sequences by their evolutionary conservation.

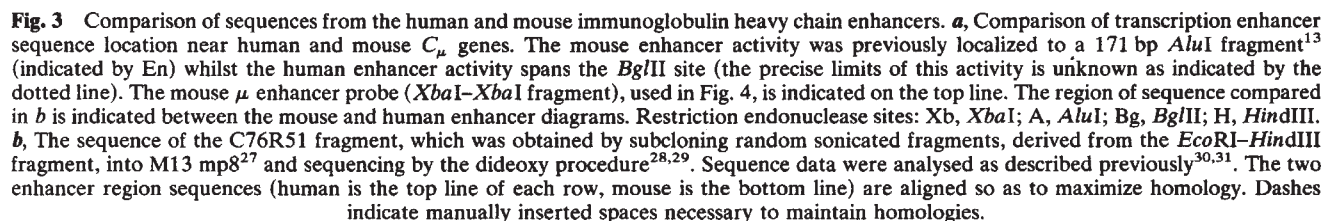


Fig. 3 Comparison of sequences from the human and mouse immunoglobulin heavy chain enhancers. **a**, Comparison of transcription enhancer sequence location near human and mouse C_{μ} genes. The mouse enhancer activity was previously localized to a 171 bp *AluI* fragment¹³ (indicated by En) whilst the human enhancer activity spans the *BglII* site (the precise limits of this activity is unknown as indicated by the dotted line). The mouse μ enhancer probe (*XbaI*-*XbaI* fragment), used in Fig. 4, is indicated on the top line. The region of sequence compared in **b** is indicated between the mouse and human enhancer diagrams. Restriction endonuclease sites: Xb, *XbaI*; A, *AluI*; Bg, *BglII*; H, *HindIII*. **b**, The sequence of the C76R51 fragment, which was obtained by subcloning random sonicated fragments, derived from the *EcoRI*-*HindIII* fragment, into M13 mp8²⁷ and sequencing by the dideoxy procedure^{28,29}. Sequence data were analysed as described previously^{30,31}. The two enhancer region sequences (human is the top line of each row, mouse is the bottom line) are aligned so as to maximize homology. Dashes indicate manually inserted spaces necessary to maintain homologies.

The Burkitt lymphoma cell line Raji (t8; 14) carries a translocated *c-myc* gene associated with the $C_{\gamma}1$ heavy chain gene⁹, with a breakpoint adjacent to an amalgamated switch sequence containing partly S_{γ} and partly S_{μ} (see this issue)¹⁹. A comparison of the restriction maps from the human immunoglobulin enhancer element and the translocated *c-myc* gene from Raji

We have shown that a functionally active transcriptional enhancer element is located ~ 5 kilobases (kb) upstream of the human C_μ gene. This may explain why V genes are only highly transcribed after they have been joined to J_H genes, even though germ-line V genes possess active promoter regions²¹. As previously pointed out, the location of the heavy chain enhancer (between J_H and S_μ) allows this sequence to be maintained near each successive C_H gene expressed during the class switch^{13,15}. So normally the $C_\gamma 1$ gene would acquire the enhancer sequence as a result of the class switch. The translocation junction of the *c-myc* gene in the Raji cell line occurs at the S region and the enhancer segment was evidently lost from the Raji 14q+ chromosome during the acquisition of the *c-myc* gene from chromosome 8. Therefore the suggestion that the oncogenesis

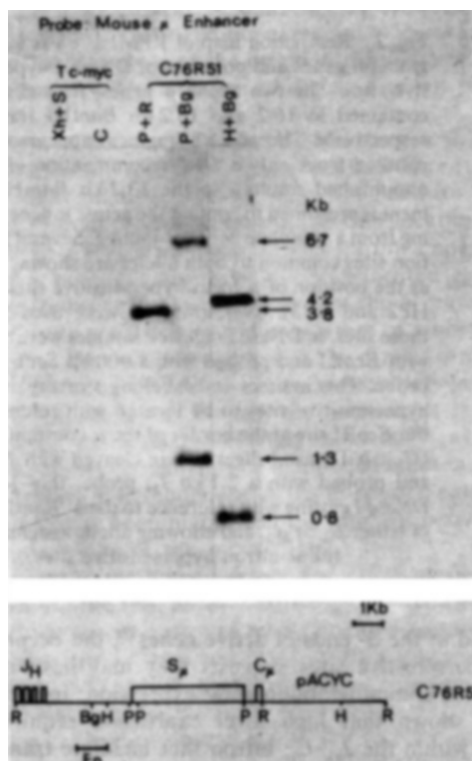


Fig. 4 Filter hybridization of normal human C_μ gene locus and Raji translocated $c\text{-myc}$ gene with a mouse immunoglobulin heavy chain enhancer probe. Clones containing the normal human $J_H\text{-}C_\mu$ locus (C76R51), or the translocated $c\text{-myc}\text{-}C_\mu$ locus from Raji cells (pUCRB19RH7), were digested with various restriction enzymes, fractionated in a 0.8% agarose gel and transferred to cellulose nitrate filters³². The filter was then hybridized as previously described⁹ with a clone M13J containing $Xba\text{I}\text{-}Eco\text{RI}$ fragment, from between J_H and C_μ , which contains the mouse immunoglobulin enhancer activity (see Fig. 3a). The three right-hand lanes contained C76R51 digests and the two left-hand lanes contained pUCRB19RH7 digests (Tc-myc). The $Xho\text{I}$, $Sac\text{I}$ and $Cla\text{I}$ sites are present in the pUCRB19RH7 clone and derive from the translocated $c\text{-myc}$ gene. Sizes were calculated from markers of λ phage DNA cut with $Hind\text{III}$. The linearized restriction map of C76R51 appears at the bottom of the figure (linearized at the $Eco\text{RI}$ site). Restriction endonuclease sites: Xh, $Xho\text{I}$; S, $Sac\text{I}$; C, $Cla\text{I}$; P, $Pst\text{I}$; R, $Eco\text{RI}$; Bg, $Bgl\text{II}$; H, $Hind\text{III}$.

in Burkitt lymphoma results from enhanced expression of $c\text{-myc}$ specifically due to its location adjacent to an active immunoglobulin enhancer appears not to be correct, at least as a general rule for the 8; 14 translocations. A similar situation also occurs with mouse myelomas. It remains a possibility, of course, that there are other enhancing elements near the C_H genes that are active in promoting $c\text{-myc}$ transcription, but these putative elements are not sufficiently active to be detected in the transient expression assay.

The breakpoint on chromosome 8 in Burkitt lymphoma is very variable: in some cases the $c\text{-myc}$ gene has a rearrangement site sufficiently close to the gene to show altered restriction fragments, whilst in many others no obvious changes can be seen. In addition, in two cases of Burkitt lymphoma cell lines with 2;8 translocations we have shown that the $c\text{-myc}$ gene does not translocate from chromosome 8 (M.O., S.M. and T.H.R. submitted). A general mechanism for the involvement of elevated levels of translocated $c\text{-myc}$ in these lymphomas is therefore difficult to imagine and it is possible that the translocation of the $c\text{-myc}$ gene has the effect of maintaining rather than elevating transcription of this gene which would normally be tightly controlled. A different, perhaps complementary, mechanism, is somatic mutation of the translocated $c\text{-myc}$ gene as indicated in the associated article¹⁹.

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DNase I hypersensitive sites in the chromatin of human μ immunoglobulin heavy-chain genes

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An immunoglobulin polypeptide chain is encoded by multiple gene segments that lie far apart in germ-line DNA and must be brought together to allow expression of an immunoglobulin gene active in B lymphocytes. For the immunoglobulin heavy chain genes, one of many variable (V) region genes becomes joined to one of several diversity (D) segments which are fused to one of several joining (J) segments lying 5' of the constant region (C) genes^{1,2}. Here we show that the rearranged μ genes of an IgM-producing human B-lymphocyte cell line exhibit pancreatic deoxyribonuclease (DNase I) hypersensitive sites in the $J_H\text{-}C_\mu$ intron that are absent in naked DNA or the chromatin of other differentiated cell types. DNA sequence analysis reveals that the major hypersensitive site maps to a conserved region of the $J_H\text{-}C_\mu$ intron recently shown to function as a tissue-specific enhancer of heavy-chain gene expression^{3,4}. A similar association of an enhancer-like element with a DNase I hypersensitive site has been reported for the mouse immunoglobulin light-chain $J_\kappa\text{-}C_\kappa$ intron⁵⁻¹⁰. These results implicate disruption of local chromatin structure in the mechanism of immunoglobulin enhancer function.

We have examined the chromatin structure of rearranged immunoglobulin heavy chain genes in the well characterized human B-cell line RPMI 1788, a μ/λ IgM producer¹¹. These cells have a diploid karyotype with two allelic μ genes, one of which has undergone a functional V-D-J rearrangement allowing production of μ chains while the other is also rearranged but to yield a D-J join (A. M. Ford, unpublished results; Fig. 1). Nuclei from RPMI 1788 cells were mildly digested with DNase I, while in parallel experiments nuclei from the myeloid line HL60 (which contains germ-line μ genes) were used as a

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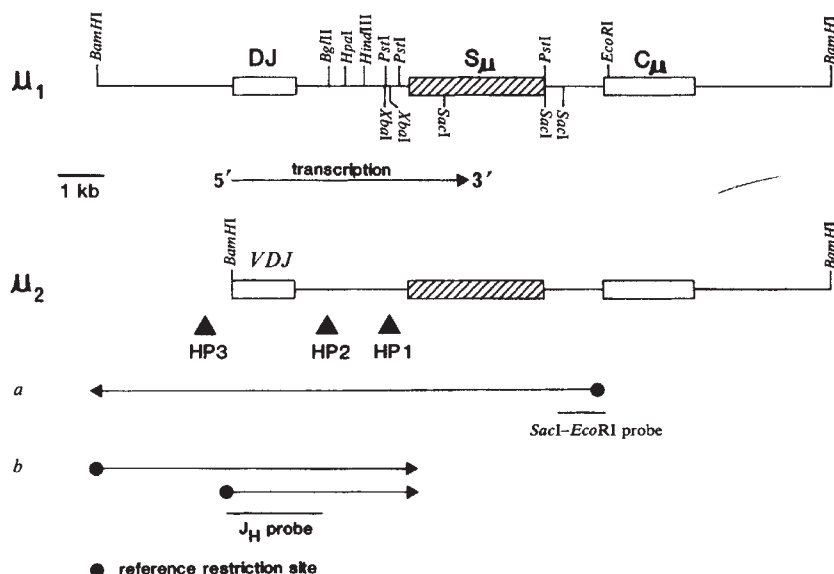


Fig. 1 Restriction map of RPMI 1788 μ immunoglobulin genes and positions of DNase I hypersensitive sites. The two allelic μ genes, μ_1 and μ_2 , are contained in 16.2 and 13.2 kb *Bam*HI fragments respectively. The 16.2 kb fragment appears to have resulted from only a *D-J* recombination (A.M.F., unpublished results), so the 13.2 kb *Bam*HI fragment is presumed to contain the active μ gene resulting from a complete *V-D-J* joining. Several restriction sites common to both alleles are shown, as well as the position of μ locus hypersensitive sites (HP1, HP2 and HP3). Two strategies were used to map these sites. **a**, DNase I digested samples were cleaved with *Eco*RI and probed with a 900 bp *Sac*I-*Eco*RI probe. This indirect end-labelling strategy¹² allows hypersensitive sites to be located with reference to the *Eco*RI site at the border of the μ constant region (C_{μ}). **b**, DNase I digests were cleaved with *Bam*HI and probed with a 2.1 kb J_H probe, thus locating DNase I cutting with reference to the 5' *Bam*HI sites of either μ_1 or μ_2 , and allowing allelic assignment of the μ intron hypersensitive sites.

control. DNA was then isolated from the nuclei, limit-digested with an appropriate restriction enzyme, and analysed by Southern transfer using an indirect end-labelling procedure¹². A restriction map of the RPMI 1788 μ genes together with the hybridization probes used here is shown in Fig. 1.

When DNA extracted from untreated RPMI 1788 nuclei was digested with *Eco*RI and hybridized to an intron-derived *Sac*I-*Eco*RI fragment, two allelic *Eco*RI restriction fragments of 12 and 20 kilobases (kb) were observed (Fig. 2a, lane 1), which are derived from alleles μ_2 and μ_1 respectively (Fig. 1). DNA from nuclei digested with increasing amounts of DNase I showed progressive disappearance of these *Eco*RI fragments and the appearance of three nuclease-generated subfragments corresponding to three DNase I hypersensitive sites within the μ genes (Fig. 2a, lanes 2-7). One site, HP3, occurs about 9.4 kb 5' of the *Eco*RI reference site (Fig. 1) and appears to lie 5' to the putative location of the *D-J* sequences (F.C.M., unpublished results). This site was not studied further.

Two other DNase I hypersensitive sites, a strongly preferred cutting site HP2 and a weaker site HP1, lie within the J_H - C_{μ} intron. From Fig. 2a, it is apparent that HP2 encompasses the intron *Bgl*II site whereas HP1 maps to the most 3' of the two intron *Xba*I sites (Fig. 1). DNase I digestion of HL60 nuclei (Fig. 2b) did not reveal the presence of hypersensitive sites in the germ-line μ genes of these non-lymphoid cells. Similarly, the germline μ genes of K562 erythroleukemia cells do not exhibit μ intron hypersensitive sites¹³. Furthermore, all three hypersensitive sites seen in RPMI 1788 nuclei are a feature of chromatin structure as they were not observed in DNase I digests of naked RPMI 1788 DNA (Fig. 2c, lanes 2-6).

To confirm the location of HP2 we used a probe that hybridizes 5' of the J_H - C_{μ} intron. *Bam*HI-cleaved DNA from the same DNase I treated RPMI 1788 nuclei used in Fig. 2a was hybridized to a J_H probe (Fig. 2d). Two strong bands, 5.4 kb and 2.3 kb in size, are those expected from DNase I cutting in the vicinity of the intron *Bgl*II site on both μ alleles (HP2). From Fig. 2d we could not unequivocally identify a band or bands arising from cutting at HP1. These would in any case be faint given the weaker preference for DNase I cutting at HP1 (Fig. 2a), and particularly if HP1 lies on both alleles.

We have determined the DNA sequence of a segment of the J_H - C_{μ} intron containing the hypersensitive sites (Fig. 3). Comparison with the mouse J_H - C_{μ} sequence using the dot matrix analysis method¹⁴ reveals an extensive, if interrupted, region of homology (Fig. 4a). Moreover the strong hypersensitive site HP2 maps to a location within this broadly homologous region.

What accounts for the presence of a strong DNase I hypersensitive site within conserved intron sequences of rearranged human μ genes? By analogy with DNase I hypersensitive sites

often found at the 5' ends of active genes¹⁵, the occurrence of intron hypersensitive sites suggests they may have a role in regulation of immunoglobulin gene expression. In fact, recent work has shown that high level expression requires DNA sequences within the J_H - C_{μ} intron that facilitate transcription of the functionally rearranged V region gene^{3,4}. Our results, with those of others^{5-10,16,17}, suggest these so-called enhancer sequences are associated with DNase I hypersensitive regions.

Enhancers were first identified in sequences near the replication origins of animal viruses such as SV40 and polyoma¹⁸. Transfection studies have shown that viral enhancers in artificial gene constructs dramatically stimulate transcription irrespective of orientation and will operate at a considerable distance (up to several thousand base pairs) 5' or 3' of the gene¹⁸. Recently both transient gene expression experiments³ and stable transfection studies⁴ using the mouse J_H - C_{μ} intron have demonstrated there is a sequence within an intron-derived 313 bp *Pst*I-*Eco*RI restriction fragment which functions as a tissue-specific enhancer, being active in B lymphocytes. Parallel studies have identified an enhancer at an homologous location in the human J_H - C_{μ} intron, that is, encompassing the intron *Bgl*II site (T. H. Rabbitts, unpublished data). Thus within the limits of these mapping results, HP2 and the enhancer sequence lie in the same region of the intron (Fig. 4a).

A more detailed comparison between the mouse enhancer region and the analogous human intron sequence is presented in Fig. 4b. These DNA sequences are approximately 68% homologous, and contain a directly repeated sequence, GTGGTT(T)TG that is highly conserved amongst the polyoma virus enhancer, the mouse immunoglobulin heavy chain enhancer, and the human μ intron sequence. The role of this element and other conserved sequences within the J_H - C_{μ} intron in determining chromatin structure and transcription remains to be discovered.

Interestingly, expression of the mouse κ light-chain gene is also dependent on an enhancer-like element contained within a highly conserved 130 bp region of the J_{κ} - C_{κ} intron^{8,9}. Induction of κ gene expression generates a DNase I hypersensitive site that has been accurately mapped to the 130 bp segment¹⁰. Deletion of this region dramatically reduces expression of κ chains when the altered gene is introduced into lymphoid cells⁸. Several groups have noted that the conserved intron region shares sequence homologies with the 72 bp SV40 enhancer, for example the sequence GGAAAGTCCCC^{7,9}. Thus, it appears that the presence of enhancer sequences in the *J-C* introns of both heavy- and light-chain genes is the basis for selective expression of the rearranged V region in B lymphocytes.

The molecular mechanism of enhancers is poorly understood. However, several hypotheses have been advanced that

Fig. 2 Lymphoid-specific hypersensitive sites in μ genes. **a**, DNase I hypersensitive sites in the μ genes of RPMI 1788 nuclei. DNase I-digested RPMI 1788 nuclei samples (see below) were limit-restricted with *EcoRI* and the μ gene fragments indirectly labelled at their 3' ends by hybridization with the 900 bp *SacI-EcoRI* probe (Fig. 1a). Samples: 1, nuclei incubated without added DNase I; 2–7, nuclei incubated with 0.71, 0.94, 1.26, 1.69, 2.25 and 3.00 $\mu\text{g ml}^{-1}$ DNase I, respectively; 8, RPMI DNA, *EcoRI/BglII* digest; 9, RPMI DNA, *EcoRI/XbaI* digest; 10, RPMI DNA, *EcoRI/HindIII* digest; 11, ^{32}P -labelled bacteriophage λ and PM2 *HindIII* fragments. Two large, major *EcoRI* fragments arise from the two different μ alleles, while three subfragments result from cutting at hypersensitive sites HP1, HP2 and HP3. By comparison of the HP1 and HP2 subfragment positions with the RPMI DNA double-digest standards (lanes 8–10) it is clear that HP1 cutting occurs near the intron *XbaI* sites, while HP2 cutting occurs in the vicinity of the intron *BglII* site. The HP3 subfragment occurs at a similar position as the λ 9.4 kb *HindIII* fragment. This locates HP3 approximately 9.4 kb 5' of the *EcoRI* reference site (Fig. 1) and places it slightly 5' to the *D-J* sequences (F.C.M., unpublished results). In our experiments, HP2 is always a strong cutting site, while HP1 and HP3 are of variable but generally weaker intensities.

b, Lack of DNase I hypersensitivity of μ genes in non-lymphoid cell nuclei (HL60). DNase I digests of HL60 cell nuclei were limit-digested with *EcoRI* and hybridized as in **a**. Samples: 1, HL60 nuclei incubated without DNase I; 2–6 HL60 nuclei incubated with 0.012, 0.036, 0.11, 1.0 and 3.0 $\mu\text{g ml}^{-1}$ DNase I; 7, ^{32}P -labelled λ *XhoI* fragments; 8, ^{32}P -labelled λ and PM2 *HindIII* fragments. Only a single large germ-line HL60 *EcoRI* fragment is observed with no obvious DNase I-generated subfragments.

c, Absence of DNase I hypersensitive site in deproteinized RPMI DNA. No DNase I hypersensitive sites were observed when RPMI 1788 DNA was analysed as in **a**. Samples: 1, ^{32}P -labelled λ and PM2 *HindIII* fragments; 2, RPMI DNA incubated without added DNase I; 3–6, RPMI DNA incubated with 0.1, 0.2, 0.4 and 0.8 $\mu\text{g ml}^{-1}$ DNase I, respectively; 7, RPMI DNA, *EcoRI/BglII* digest; 8, RPMI DNA, *EcoRI/XbaI* digest; 9, RPMI DNA, *EcoRI/HindIII* digest; 10, ^{32}P -labelled λ and PM2 *HindIII* fragments. **d**, Allelic assignment of the μ intron hypersensitive site HP2. DNA samples from the same RPMI 1788 nuclei digest series used in **a** were limit *BamHI* restricted and hybridized with a J_H probe (Fig. 1b), in order to assign J_H - C_μ intron DNase I cutting with reference to the 5' *BamHI* sites of the two μ alleles. Samples: 1, ^{32}P -labelled λ and PM2 *HindIII* fragments; 2, RPMI DNA, *BglII* digest; 3, RPMI DNA, *BglII/BamHI* digest; 4, RPMI nuclei incubated without added DNase I; 5–9, RPMI nuclei digested with 0.71, 0.94, 1.26, 2.25 and 3.0 $\mu\text{g ml}^{-1}$ DNase I, respectively, followed by limit digestion with *BamHI*; 10, RPMI DNA, *HindIII/BamHI* digest; 11, RPMI DNA, *HindIII* digest; 12, ^{32}P -labelled λ and PM2 *HindIII* fragments. Two DNase I-generated subfragments are visible, consistent with DNase I hypersensitive sites in the J_H - C_μ introns of both alleles. Because the smaller subfragment is similar in size to the μ_2 allele 5' *BamHI-BglII* fragment (small band, lane 3) and substantially smaller than the μ_2 5' *BamHI-HindIII* fragment (small band, lane 10), this subfragment appears to result from hypersensitive site cutting near the *BglII* site of the active μ_2 allele, corresponding to HP2 in **a**. The larger fragments in lanes 3 and 10 are too small to be μ_1 allele *BamHI-BglII* and *BamHI-HindIII* fragments (Fig. 1), and therefore appear to be μ_1 5' *BglII-BglII* and *HindIII-HindIII* fragments, respectively. Thus these fragments cannot be compared with the larger DNase I-generated subfragment. However, the size of this subfragment (~5.4 kb, as judged with its co-migration with the 5.4 kb marker bands in lanes 1 and 12) is consistent with HP2 cutting near the μ_1 J_H - C_μ intron *BglII* site (μ_1 5' *BamHI-BglII* distance is 5.2 kb, Fig. 1).

Methods: RPMI 1788 and HL60 cells were grown in RPMI 1640 medium supplemented with antibiotics and 10% fetal calf serum. Nuclei were prepared and digested with DNase I essentially as described previously^{21,22}, but with the inclusion of 0.1 mM phenylmethylsulphonyl fluoride in all buffers. DNase I digestions on deproteinized RPMI DNA were performed similarly. After digestion samples were made 0.2% w/v in SDS and 10 mM EDTA, and the DNA was purified as described⁴. DNA samples were limit digested with either *EcoRI* (parts **a**, **b** and **c**) or *BamHI* (**d**), phenol extracted, precipitated with ethanol, and dissolved in distilled water. DNA samples (15 μg) were electrophoresed in 0.7% agarose gels at 5 V cm^{-1} for 40–60 h in a buffer containing 36 mM Tris, pH 7.8, 10 mM NaH_2PO_4 , 1 mM EDTA, and ethidium bromide (0.5 $\mu\text{g ml}^{-1}$). After electrophoresis, the DNA was transferred to nitrocellulose²³. Transfers were hybridized with either a 900 bp intron *SacI-EcoRI* fragment (for *EcoRI* digests), or 2.1 kb J_H fragment extending from an artificial *EcoRI* site 5' of J_H 3 to the μ intron *BglII* site (for *BamHI* digests). Both fragments were isolated from the J_H - C_μ intron subclone C76R51, labelled by nick translation using [α - ^{32}P]dCTP and hybridized essentially as described in ref. 6. Autoradiography was for 40–200 h at -70°C using preflashed Kodak XRP-5 film with two Dupont Kronex intensifying screens sandwiching the film and transfer. Numbers at right-hand side are marker sizes (in kb).

accommodate the ability of enhancers to exert regional transcriptional control irrespective of their orientation and location relative to a nearby gene. One attractive suggestion¹⁸ is that enhancer sequences act as entry points for RNA polymerase and/or other transcription factors. Enhancers lie in DNase I hypersensitive regions, consistent with the idea that they are involved in opening up the local chromatin structure thus making it more accessible to the transcriptional apparatus. As immunoglobulin enhancers act in a tissue-specific fashion, additional lymphocyte factors are implicated in immunoglobulin gene activation. The identification and characterization of these specific components, most likely proteins that recognize the enhancer DNA sequences, should allow further elucidation of immunoglobulin gene regulation.

Finally, Storb *et al.*¹⁹ have broached the possibility that DNase I hypersensitive sites within the J_H - C_μ intron may reflect the potential of T and B cells to undergo switch recombination, that is, the replacement of the *C* gene of one class with that of another. Precedent for this idea is provided by the yeast mating type locus, in which DNA sequences that participate directly in mating type switch recombination reside in DNase I hypersensitive sites²⁰. In this regard we note that HP1 maps adjacent to the human switch recombination sequences (Fig. 1).

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10 20 30 40 50 60 70 80 90 100 110

ATTATTATGAGCGAGCTGGAGCCAGATGATGATTATGAGCTCAGATGGCTGCATGGGGCTCTCCGACCCACAGCGAGTGGCAGAGCGAGGTCAACCGAGAGCTATTATTAGAGAG 120

Bgl II
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TAGGCGAGGCTGACCGAATCTGAAGAGCTTTTTTAACTATCTGAATTTTCATTTCTAGCTTATCACTGCTAGTTGTGCAACAGCATATCACTTCTTAACTGCATTCAT 360

TTTTTAAGTAGATGTTTAAAGAAATTAACAGCTCTAGGAGAGTATTAAGCTATCTCAAAAAGTTTTTTTAAATTAGCTGTGTATCCCTCATGTGATTAATCTCAATACTTTTT 480

Hpa I
CGATACCTCAGAGCATTTATTTCTAATTAAGTCTGTCTCAACATCTTTTTAGGTTAACTCGTTTTCTCTTGTGATTAGAGGAAACACTTGTATATCTGATAGTGTGGCTTCATTTTA 600

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CCCGGCTAGGCTTTGGGTCTCCCGGACTACCGAGAGCTGGATGCTGCTCTGCTCCGCGGAGCTGCTGCTCAGGCCCCAGCCCTGTTAAATGCACTGGAGGATGATTTCA 960

Hind III
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CCTTAAAGTAGCCCATGCTTTCCAAAGCGATTTATGGTAATAGGCGAGATTTTAAAGTGGCAATTCAGTAATTAATGCTATTTCTGTTGTTTCCATGATGACTGTCTTAGAGGAA 1440

Pvu I *Xba* I
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GATTTGATATGCTTTGGAAACCAACCTAGTGGGCTGGGCGAGATCGC 1731

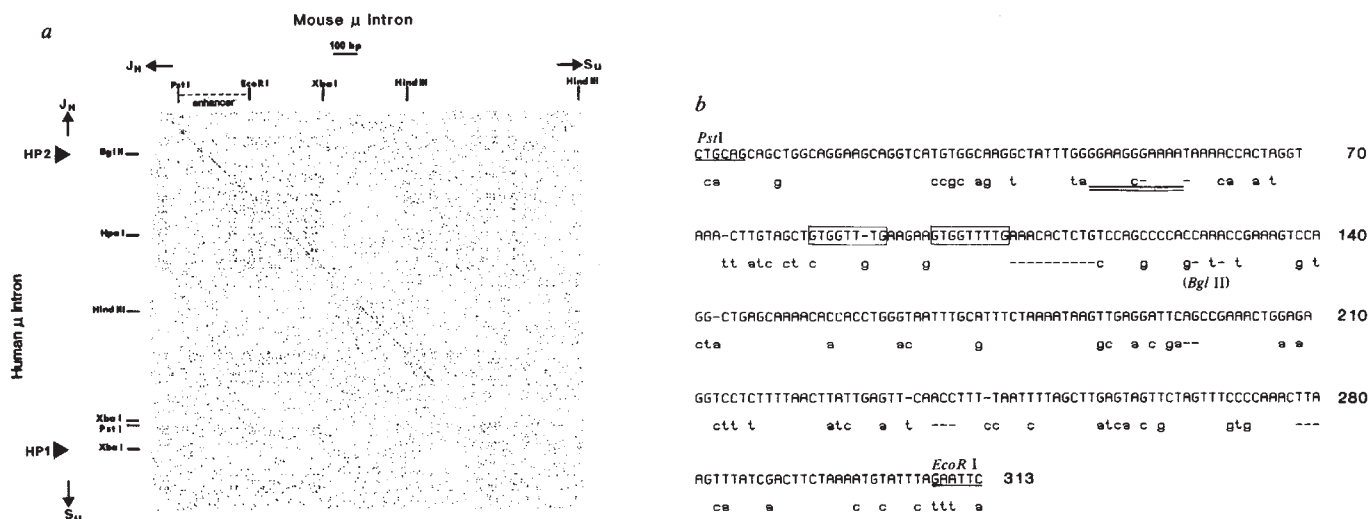


Fig. 4 Analysis of the μ hypersensitive site sequence. **a**, Dot matrix comparison of human and mouse μ intron sequences^{3,4,25} 5' to 3' using a filter five dot matrix computer algorithm, in which a dot indicates five contiguous matching bases between the two sequences, and a 45° diagonal indicates an homologous segment¹⁴. An extensive homology region of about 1,200 bp covers the sequence between the two intron hypersensitive sites HP1 and HP2, and includes HP2. The position of the mouse heavy-chain enhancer is indicated by a dotted line. **b**, Alignment of the mouse immunoglobulin heavy chain enhancer sequence with the analogous human J_H - C_μ sequence in the vicinity of HP2. A 313 bp sequence found to be critical for the enhancer activity of the mouse J_H - C_μ intron^{3,4} is aligned with a sequence from a similar position in the human μ gene. The two DNA segments show an approximate 68% homology (counting both insertion/deletions and altered bases as mismatches). The mouse sequence is shown in upper case letters, with the human sequence, where different, indicated by lower case letters beneath the mouse sequence. Dashes indicate deletions in either sequence. The *Pst*I and *Eco*RI sites bounding the mouse enhancer are underlined, and the position of the human intron *Bgl*III site is indicated in parentheses. Numbers at the right-hand side of the figure indicate distances from the mouse *Pst*I site. Boxes highlight two direct repeats of the consensus enhancer sequence GTGGTT(T)TG, one of which (GTGGTTTTTG) is exactly conserved between the human sequence and the mouse and polyoma virus enhancers^{3,4,26}. There is double underlining beneath a second sequence of 11 bp (AGGAAGCAAAA) which is exactly conserved between the human J_H - C_μ intron and the polyoma enhancer, where it is found adjacent to the GTGGTTTTTG segment.

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Bones, stones and Buckland

Roy Porter

The Great Chain of History: William Buckland and the English School of Geology (1814–1849).

By Nicolaas A. Rupke. Clarendon: 1983. Pp.322. £22.50, \$45.

In the heroes versus villains jousts which have traditionally dominated histories of English geology, the "Diluvialist" school of the early nineteenth century has always had a bad press. In contrast to the "great tradition" rising from Hutton through Lyell and beyond, geologists such as William Buckland, the Conybeare brothers and Daubeny have been depicted as black sheep, retrogressive and wrong. After all, in opting for neptunism rather than plutonism, diluvialism not fluvialism, and leaning towards cataclysmic rather than actualistic geomorphological agency, they backed the wrong horses. Worse, in championing catastrophism they were betraying science by grinding theological axes. William Buckland, later Dean of Westminster, pronounced that geology which did not ascend to religious truth was unsatisfactory; and in his *Reliquiae Diluvianae* (1823: *Relics of the Deluge*) argued that bone-cave evidence gave "decisive and incontrovertible" proofs of his historicity of Noah's Flood. In doing so, he committed the sin against Bacon of unwisely mingling Genesis and Geology; worse, he finally ended up with egg on his face, being forced to retract some years later in the decent obscurity of an ignominious footnote. But historians have always seen Buckland — with his giant bone-bag, scientific clowning and fascination with coprolites — as a bit of a buffoon.

In his erudite work of revisionism, Nicolaas Rupke shows that this reading of the Oxford Diluvialists is largely caricature, foisted upon them by opponents such as Lyell, and accepted uncritically ever since. Of course, there is no denying that they aimed to harmonize their fieldwork with Christian Creationism; but, Rupke asks, how else was Buckland, the first Reader in Geology, to establish the science in pre-Reform Oxford, still exclusively Anglican and dominated by the clergy? The new science had to be grafted upon old scholasticism, the Book of Nature reconciled with both Scripture and written historical records. In any case, the religion of Buckland and his friends was not dogmatic but liberal, sweet reason itself compared to the obscurantist Evangelicals and Tractarians who sniped at the emergent science. Nor did their theology blinker their science; it rather suggested valuable geological perspectives, such as a search for origins, and an eye for the adaptation of organism and environment. Such outlooks stimulated them to plot the sequence of the strata, and reconstruct

extinct flora and fauna using comparative anatomy; on this basis they made valuable contributions towards recovering the ecology of lost worlds and understanding the laws of geological change.

Orientated thus, Buckland's Oxford School formed the headwaters of what was to become mainstream Victorian historical geology. Even their soft spot for cataclysmic agency could prove fruitful. It was no accident that Buckland, one-time prime devotee of the Deluge, became Britain's first and foremost convert to Agassiz's glacialism and theory of Ice Ages. In view of all this, Rupke argues, we need to view the dynamics of geology not as a series of encounters in which truth vanquished error, but as the conflux of many different schools, each contributing its own insights; amongst these, this "English School" takes its place alongside the Scottish (Huttonian), the German (Wernerian) and the French (Cuvierian).

Rupke's rehabilitation is generally persuasive, though certain doubts remain. For one thing, he himself occasionally counters caricature with caricature. In rescuing Buckland from Lyell's tarbrush, he has sketched a one-sided portrait of Lyell, oversimplifying him into an "a priori" and "doctrinaire" Uniformitarian. Then take his contention that the essence of Oxford geology lay not in religious catastrophism but in its commitment to historical geology. It is an important corrective. But it skims somewhat over two facts. First, before the 1830s, English geology was probably more orientated towards descrip-

tive stratigraphy than to reconstructing Earth history. Second, Buckland and his followers did indeed believe that science proved the crust had undergone stupendous convulsions (the term "catastrophism" was coined after all by their sympathizer, William Whewell). Right to the end of his career, Buckland felt confident that science pointed to divine interventions (it was the "clear design of Providence", he thought, by strategic placement of mineral resources, to make Britain "the most powerful and richest nation on earth"). Indeed, it was Buckland who had been largely responsible for making the Deluge a respectable topic of scientific inquiry.

Before Buckland, the elite of British geology had been keeping judiciously silent about Noah's Flood. By contrast Buckland's rash confidence opened a Pandora's Box, whose outcome was the protracted sterile controversy about natural law and divine miracles which dogged English (but not Continental) natural history for the next generation. This is one reason why we must be wary of Rupke's tendency to conflate Oxford geology with English geology. There were numerous other types of contemporary researchers including surveyors such as William Smith, and the Geological Society of London elite, and most of these viewed geology in a more secular light.

Rupke has cleverly crafted his book as a historical nest of three boxes: a broad conspectus on English geology, which contains a close-up of the Oxford School, enclosing finally a portrait of Buckland. His view of Buckland as the key to English geology in the generation before Lyell is just, and makes one hope he will one day give us a biography of this engaging and misunderstood pioneer. □

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REASONS

The savants eat out — dinner in the model of *Iguanodon* at the Crystal Palace, near Croydon in south London, on 31 December 1853.

From antiquarianism to archaeology

Stuart Piggott

The Establishment of Human Antiquity.

By Donald K. Grayson.

Academic: 1983. Pp.262. \$27.50, £18.20.

It has become a commonplace in histories of archaeology and evolutionary theory to point to the year 1859 as the moment at which, in England, the biological hypotheses of Darwin, and the archaeological and geological inferences drawn from the association of stone tools and an extinct fauna in the Somme gravels, coalesced to form a joint demonstration of the antiquity of man. But when the detailed history of the ideas involved is examined, as Grayson has now done, the two can be seen as almost coincidental, with neither dependent on the other, but accidentally published at the same time.

The study of the artifacts of extinct human societies which was to become archaeology, made a promising start in the late seventeenth century as part of the empirical and taxonomic approach to natural phenomena. Stone tools and weapons were separated from fossils and minerals, and accommodated in a model of the past based on ethnographic parallels as representing an ancient barbarian state of culture of unknown antiquity. From the 1720s however, interest waned as antiquities became regarded as ends in themselves rather than potential evidence from which to infer the nature of a pre-historic past.

No significant development, conceptual or in field technique, took place until the second half of the nineteenth century. In geology and palaeontology it was quite different; the principles of stratigraphy and sequential ordering were early established in a broad sense, though the complex microstratigraphy of Pleistocene and Holocene deposits where both natural and man-made agencies might be combined (as in inhabited caves) presented peculiar difficulties, as indeed they do today.

In France, Boucher de Perthes's discoveries of extinct animals and stone tools in open stratified deposits, published in 1847, met with an unfavourable reception from the scientific establishment: it is good to have in this book a detailed survey of the Continental scene with its jealousies and in-fighting between individuals and institutions. But in 1859 the classic Anglo-French meeting at Abbeville took place, between Boucher, two senior and distinguished English geologists, Joseph Prestwich and Hugh Falconer, and a young paper manufacturer in his mid-thirties who deserves the title of the first English archaeologist rather than antiquary, John Evans. Geology and archaeology combined in the alliance so long needed, and the antiquity

of man could now be approached through these two disciplines, in parallel with the concept of biological evolution and comparative anatomy.

Wholly theoretical schemes of the extended development of human societies from savagery to civilization had been put forward in eighteenth century Scotland and France without reference to archaeological or geological evidence, and by 1848 the Danish Stone-Bronze-Iron Age technological sequence was available in English. Further synthesis was now inevitable, with the data against which models of the past could be tested, scientifically established. A "conflict of science and

religion" at this time has been exaggerated and over-dramatized: as Grayson puts it "the biblical chronology as applied to the human species had become an issue that could no longer be compellingly addressed in a scientific fashion", and ceased to be relevant in a climate of opinion increasingly unfavourable to its acceptance. This book admirably surveys the complicated interaction of social, intellectual and emotional factors which brought such a climate into being. □

Stuart Piggott retired as Abercromby Professor of Archaeology at the University of Edinburgh in 1977.

The pen is mightier than the endoscope

Walter Gratzer

The Language of Medicine: Its Evolution, Structure, and Dynamics, 2nd Edn.

By John H. Dirckx.

Praeger: 1983. Pp.193.

\$22.95, £22.50.

A Treasury for Word Lovers.

By Morton S. Freeman.

ISI Press, Philadelphia: 1983. Pp.333.

Hbk \$19.95; pbk \$14.95.

THE dust-jacket of Dr Dirckx's book induces a certain unease in the non-medical reader, for it bears a warm commendation from an organ called *Gastrointestinal Endoscopy*, and indeed the book fell open for me at a dissertation on patients' malapropisms, in which it is recorded that in France *hémorroïdes* are commonly transformed into *émeraudes*, or emeralds. (This brought to mind the fate of the Philistines, whom the Lord smote with a plague of emerods in their private parts: I believe the view of medical historians is not that the Philistines succumbed to an epidemic of piles, but rather that they were visited by the plague and that the emerods were buboes.)

Elsewhere Dr Dirckx (a practising doctor, who runs a university clinic and plies his stethoscope in Dayton, Ohio) descants in similar vein on euphemisms, and the successive strata of propriety with which they in turn are covered up. He recalls Don Quixote's instruction to Sancho Panza that he should eschew the vulgar "burp" (or *regoldar* in Spanish, from the Latin *regurgitare*) in favour of "eruct" (*erutar*). He tells us that the term *infarct* acquired its redundant *c* out of misplaced delicacy, and that the Italian term, which is classically correct, is *infarto* and the Spanish *infartación*.

But I should not give the impression that *The Language of Medicine* is scatological or even to any large extent gastrointestinal: it is in fact a sustained *tour de force* of

polyglot erudition, written with style and relish, a constant pleasure to read and irresistible for browsing. You will discover, for instance, that *placebo* evolved from the text of a psalm (114), which concludes in the Vulgate: "*Placebo Domino in regione vivorum*" (I shall be pleasing unto the Lord in the land of the living), and introduces the Office for the Dead in the Latin rite. *Placebo* became a general expression for obsequiousness and servility, and in due course for a medicine designed to please more than cure. Dr Dirckx notes that *lavabo*, which also occurs in a psalm, acquired its modern meaning of lavatory by a parallel route. Or were you aware that the disrespectful use of the word *leech* to denote the medical man, rather than the worm, has a degree of etymological respectability (although Dr Dirckx questions its validity), for it was, it seems, in current use in Middle English and derives from the Anglo-Saxon *læce*, being mirrored in the modern Swedish *läkare*, meaning doctor? Well, neither I confess did I, nor yet that the Old English for the common leech, *Hirudo officinalis*, was *officinal*; this fell into disuse, Dr Dirckx conjectures, because subeditors of former days were no different from their descendants and tended to delete the *n* and transmute the word into one that they knew.

Dr Dirckx is no less compelling on the tensions between Anglo-Saxon and classical derivations than on the mutilation of the latter in modern times. He shows that the divide between technical and demotic linguistic usage endures today and has perhaps widened. Yet here and there technical terms have flowed back into the common language and enriched our vocabulary; *sibling* for example is a word long wanting in English (compare the German *Geschwister*), and I am sure I have seen a stripper referred to as an *ecdysiast*. Two earlier examples that occur in the book are *plethora* and *hectic*, both medical terms, discarded by the profession and left to journalists.

Dr Dirckx is not in favour of the kind of patriotic purge of classical derivations that occurred in Germany, where paediatrics is *Kinderheilkunde* (and for that matter a

telephone is a farspeaker, a vacuum cleaner a dustsucker and a bra a bosomholder). Nor is he distressed by mixed derivations, which he holds to be inescapable, but he inveighs magisterially against the reckless and classically illiterate coinages that are tossed in growing profusion into the medical (and scientific) literature, and left there to fester.

The Language of Medicine contains besides much else, an entertaining section on the names of commercial products (though this is an area that could be quarried for a learned volume all on its own: according to E.S. Turner's history of advertising, there have been, to take only one instance, not less than sixteen trade-names for phenolphthalein alone). There is a richly diverting chapter on the argot of doctors, patients, nurses and typists, and another, with the title "Diseases of the Tongue", that treats of the abuse of language and contains some examples of modern medicalese so monstrous they have a certain majesty. If it pleases you to know that *hysteria* means womb-trouble, that *influenza* comes by way of stellar influence, that *testis*, meaning witness, recalls the early custom of placing the hand over the testicles when taking an oath, and that *Botulinus* derives from *botulus*, a sausage, then you should seek the means to possess this beguiling book, even to the extent of laying out hard cash.

A Treasury for Word Lovers is not of the same calibre. It is a collection of short essays, one or two to a page, mostly on vogue words and others frequently misused. There is sound instruction for the less literate reader, who might however find what he needs more readily in something on the lines of Room's *Dictionary of Confusibles*. Mr Freeman is no Thurber or Mencken, but he writes pleasantly, and a random trawl brought up some agreeable items. Tested on some common modern solecisms, the book scored well enough. It is excellent on *that* and *which*, for instance, and properly excoriates the illiterate usage of *hopefully*, with several examples but no indication of how this curious aberration has infiltrated the language. (My own surmise is that it originated in German-American *emigranto*, or *Hyman-Kaplanese*, in which *hoffentlich*, rather than *hoffnungsvoll*, was rendered as "hopefully".) Mr Freeman denounces the horrors of modern media prose wherever he encounters it. He gives no examples as arresting as the words of a New York broadcaster not long ago, who witnessed an accident, in which a helicopter plunged into the East River. "We have," he said, "a zero-survivability situation". We should be grateful to the likes of Dr Dirckx and Mr Freeman for standing between the English language and a like predicament. □

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The long shadow of Lysenko

Edward N. Trifonov

Principles of Modern Genetics, 2nd Edn.
By S.M. Gershenson.
Naukova Dumka, Kiev: 1983. Pp.560.
Price not known.

THE gradual transition from classical to molecular genetics during the 1940s and 1950s was a time of painful rebirth for Russian geneticists. Most of them, Mendelians, had been repressed, and in many cases virtually condemned to death in labour camps. Sergei Mikhailovich Gershenson was one of the few who somehow reappeared alive.

Gershenson's early studies on the sex-chromosomes of fruit-flies (1928–1935) are still occasionally discussed in books on classical genetics, but his interests today are mostly in the field of molecular biology (of insect viruses, specifically). Probably because of this, his textbook is comprehensive and neatly balanced between classical and molecular genetics.

It soon becomes clear that the book, which is in Russian, is written by an excellent lecturer — one can almost feel the passion of a committed teacher as the author carefully covers a complexity of factual information, leading to exact concluding formulations at the end of each chapter. The first half of the book displays the key ideas of genetics, both in their original and their modern context. Mendelian laws are elegantly introduced via green and yellow *Chlamydomonas*, thus postponing the traditional statistics of Mendel's peas. Chromosomal theory is then unravelled, bringing the reader logically rather than chronologically to the revelations of molecular biology. All basic

terminology, conveniently picked out in bold type, is introduced sequentially and is well explained; Gershenson has evidently taken great pains over the continuity of his account. Nor is the text ever dull, punctuated as it is by relevant anecdotes — one example is the story of a simple-minded gardener who selected seeds of pink flowers to plant all over town; sure enough, the resulting blooms showed segregation to red and white.

The rest of the text is an introduction to more specialized branches of genetics and molecular biology, and includes quite recent developments in genetic engineering, immunogenetics, transposons, splicing and oncogenes.

Somewhat unusual for this kind of book is the inclusion of chapters on non-heritable modifications, ontogenesis, evolution and practical selection. It is difficult to escape the feeling that these sections are the long delayed echoes of times when Russian genetics was under criminal investigation. For example, Lysenko appears in the introductory chapter as a scholar whose theories just happened to be wrong. One can only guess at what actually happened from a few passages such as "Genetic studies in the USSR revived after a period of decline . . ." or "Genetics is taught in all Universities . . ." and "The All-Union N. Vavilov Society of Geneticists and Selectionists . . . broadly propagates achievements of genetics" (Vavilov died in prison in 1943). It is hard to say whether the author chose not to discredit Lysenko, or was prevented from doing so by censorship; probably both, in a diabolically concerted way. Apart from such aberrations, however, this is a textbook of high quality, one which bears comparison with its Western counterparts. □

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Sea views

P.M. Holligan

Oceanography: The Present and Future.
Edited by Peter G. Brewer.
Springer-Verlag: 1983. Pp.392. DM 110, \$45.50.

A SYMPOSIUM "Will We Use The Oceans Wisely — The Next Fifty Years in Oceanography", held at Woods Hole, Massachusetts in 1980 to mark the fiftieth anniversary of the founding of the Oceanographic Institution, forms the basis of this book. It consists of twenty-two short articles organized into sections on small-, regional- and global-scale oceanography, as well as the human scale of resources and communications. Authors from a diverse range of disciplines provide authoritative summaries of present work, speculation

about the future and, in some cases, anecdotal accounts of the past which make the whole book most enjoyable. It succeeds well in conveying the interdisciplinary nature of oceanography and the problems of investigating processes over very wide temporal and spatial scales.

The editor regrets the omission of the geological and geophysical contributions from the book. It also seems a pity that some general account of biological production could not have been included, especially as recent progress in understanding the interaction between biological activity and the geochemical cycles is already leading to new lines of research.

Three consistent themes emerge in the book. First, rapid developments in instrumentation and techniques of data analysis have led to an immediate need for innovative scientists to investigate oceanic processes by new methods such as direct velocity measurements and remote sensing.

Secondly, an even more integrated approach between disciplines is required to develop a coherent view of the oceans which can be related to other studies of the total environment. As J.H. Steele, the present director at Woods Hole, writes in the final chapter, "Earth Sciences" must become the broad conceptual unit within which oceanographers, geochemists, meteorologists etc. approach the practical problems of the future; in this context the role of big institutions is vital, both in leading the way and in providing facilities for outside people to participate in research. And thirdly, there is wide agreement that the oceanic environment needs protection now. There is good evidence that human impact on the oceans

represents a "global geochemical experiment" with unknown consequences (p.305), and equally worrying are the various shoreline problems typified by the New Jersey beach scene (p.98). More optimistically, however, the fishery and aquaculture experts give considerable hope for better manipulation and exploitation of biological resources provided pollution is controlled.

Apart from the upside down satellite image of the Gulf of Maine (p.70), the production of the book is excellent. It should be read by both oceanographers and earth scientists. □

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Animals and plants

J.B. Whittaker

Herbivory: The Dynamics of Animal-Plant Interactions.

By Michael J. Crawley.
Blackwell Scientific/University of California Press: 1983. Pp.437. £27.50, \$45.

PREDATORS have enjoyed much more attention from textbook writers than have herbivores, perhaps because it is easier for a single author to appreciate interactions within a kingdom than between animals and plants. It was also easier to model the dynamics of animal-animal interactions, at least until the breakthrough of Harper's *Population Biology of Plants*, published by Academic Press in 1977. Up to then, investigators of the three groups concerned in herbivory — vertebrate herbivores, invertebrate (mainly insect) herbivores, and plants — had largely gone their respective ways; such grazing experiments as were conducted were usually confined to large, easily excluded vertebrates, while studies of invertebrate herbivory, led by entomologists, tended more towards explaining how plants affect animal feeding and dynamics than vice versa.

Bringing together these viewpoints and attempting a balanced synthesis is a formidable task. Dr Crawley has chosen to tackle it by emphasizing the dynamics of interactions between species in three ways: by first showing us how plants might be affected by grazing animals; by next discussing the extent to which the dynamics of herbivore populations might be determined by their food and the problems of

obtaining it; and, thirdly, by bringing together these two themes, mainly in the form of population models. The last section of the book deals with community dynamics.

Within this framework, this is a stimulating book which follows the fashionable approach of dealing with ecological principles and processes in terms of generalized hypotheses supported by selected references (about 1,000 in this case, though with a few curious omissions of recent important work and of some older yet still pertinent reviews). All this has been fused into approximately 350 pages of ideas and information which cover an extremely wide array of subject matter. The technique used is one of illustrating a rapid and vigorous flow of ideas with brief descriptions of original studies. The drawback is that this sometimes reads like a series of abstracts which tells us little about the organisms themselves. I jotted down a list of over a dozen well-known, critical studies of herbivores which were together referred to in a total of five pages, in only one case with any continuity of discussion.

Valuable as they are, syntheses from hundreds of references cannot provide a complete substitute for detailed case studies which are the real starting point for so many of the ideas so enthusiastically discussed. For this, perhaps idiosyncratic, reason, I would recommend the book to experienced workers as an essential and comprehensive review. But for undergraduates, to whom syntheses of ecological information can seem beguilingly simple, I would caution the need for more direction towards substantial field studies which alone can put theories into perspective by showing how general principles may interrelate in the dynamics of one system. All readers, however, should find the summary chapter most useful and thought-provoking even if, as the author suggests and I agree, they will not necessarily concur with all the conclusions. □

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Agents of change

C. Tease

Aneuploidy. By D.J. Bond and Ann C. Chandley.

Oxford University Press: 1983. Pp.200. £25, \$69.

DELIBERATE or accidental exposure to radiation, chemicals and drugs is commonplace in the human population and concern has been expressed that some at least of these agents may increase the risk of chromosomal imbalance (aneuploidy) in subsequent generations. This possibility stimulated great interest both in the mechanisms causing aneuploidy and in experimental approaches to characterization of the effects of suspect agents.

Bond and Chandley's review of aneuploidy examines the current understanding of these topics. With regard to human aneuploidy, they discuss, for example, the importance and possible causes of the maternal age effect and describe relative chromosome involvement in spontaneous aneuploidy as determined from abortuses. More usefully, the various suggested mechanisms leading to aneuploidy, ranging from the obvious (non-disjunction) to the more speculative (extra replication of chromosomes), are discussed fully in the light of experimental results. It is salutary to be reminded that the relative contribution of each putative mechanism to spontaneous aneuploidy has not yet been determined.

A substantial portion of the review is given over to summaries of results from experiments on the effects of physical and chemical agents inducing aneuploidy in a diverse array of test organisms. Radiation exposure has been shown to induce aneuploidy in a number of species. The larger part of the data derives from *Drosophila* reflecting the long interest in radiation effects in this species. The causes of radiation-induced aneuploidy have yet to be unequivocally determined although much evidence has been accumulated to favour the view that it is the result of the induction of chromosome interchanges. Chemical agents have not been studied to the same extent as radiation. The observation that certain compounds show conflicting behaviour with regard to aneuploidy induction in lower and higher eukaryotes is disappointing given the relative ease of using such lower eukaryotes as fungi in screening programmes.

Overall, Bond and Chandley have provided a thorough yet concise synthesis of approaches to aneuploidy. Their book will be of great value to those engaged in such research and should prove of interest to anyone concerned with aneuploidy in the human population. □

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New in paperback

DAVID Bohm's *Wholeness and the Implicate Order* (reviewed by Abner Shimony in *Nature* 291, 435; 1981) has recently appeared in paperback. Publisher is Ark Paperbacks, an imprint of Routledge & Kegan Paul; price is £2.95, \$6.95.